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X-RAY CRYSTALLOGRAPHIC STUDIES OF  
OUABAIN, A CHLORINE-CONTAINING TERPENE  
AND THREE SULFONIUM PERCHLORATES

by



WILLIAM BERTRAM THOMAS CRUSE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH  
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## ABSTRACT

The crystal and molecular structure of ouabain tetrahydrate was determined by X-ray diffraction methods. The sterol is joined to the L-rhamnopyranose moiety by an  $\alpha$ -glycosidic bond. The conformation of the steroid is similar to that observed in digitoxigenin except that the butenolide ring is rotated  $164^\circ$  about the C(17)-C(20) bond. The crystal contains four water molecules of crystallization which exhibit varying degrees of water-methanol disorder. The crystal is held together by a complex network of intermolecular hydrogen bonds. A water molecule is hydrogen bonded to the lactone carbonyl oxygen atom. An intramolecular hydrogen bond is observed between the  $5\beta$ -hydroxyl hydrogen atom and the oxygen atom bonded to C(1) as well as between the  $11\alpha$ -hydroxyl hydrogen atom and the oxygen atom bonded to C(19).

The spatial disposition of the 3-hydroxyl, 7-keto and ester carbonyl oxygen atoms in a model of the cassaine molecule was observed to mimic the spatial disposition of the  $3\beta$ -hydroxyl, 14-hydroxyl and lactone carbonyl oxygen atoms in cardiac glycosides only when models of the latter were given the conformation observed in the ouabain tetrahydrate crystals. This conformation may correspond to the 'active' conformation of the cardiac glycoside molecule as it binds to the  $[\text{Na}^+, \text{K}^+]$ -ATPase receptor.

The crystal and molecular structure of gutierolide ( $\text{C}_{21}\text{H}_{31}\text{O}_5\text{Cl}$ ), a chlorine-containing diterpene isolated from Gutierrezia dracunculoides (DC) Blake, was determined by X-ray diffraction methods. The molecule exhibits two cis-fused chair-conformation cyclohexane rings which are flattened at the ring junction. An intramolecular hydrogen bond is





observed between the hydroxyl hydrogen atom and the axial oxygen substituent bonded to the cyclic acetal.

The crystal structures of

(A) (d,l)-4-nitrobenzylethylmethylsulfonium perchlorate,

(B) (l)-3,5-dimethyl-4-methoxybenzylethylmethylsulfonium perchlorate and

(C) (d)-4-chlorobenzylethylmethylsulfonium perchlorate

were determined by X-ray diffraction methods. The average carbon-sulfur bond distances in A, B and C are 1.810; 1.806 and 1.811 Å respectively. The C-S-C bond angles are dependent on the number and magnitude of the steric interactions among the three substituents bonded to sulfur. The distance of the sulfur atom from the plane of the three  $\alpha$ -carbon atoms is 0.767(1) Å in (A), 0.804(1) Å in (B) and 0.756(1) Å in (C). The asymmetric sulfur atoms in (B) and (C) have the (R)-configuration based on the anomalous dispersion of sulfur and chlorine.





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PREFACE

Symbol designations and formulae used frequently within this thesis.

|                |  |
|----------------|--|
| $h, k, \ell$   | Indices of the reflection from a set of parallel planes  |
| $\theta$       | Bragg angle of the reflection  |
| $a, b, c$      | Unit cell edge lengths   |
| $F_{hkl}, F_h$ | Structure factor for the unit cell corresponding to the Bragg reflection ( $hkl$ )   |
| $F_o$          | Observed structure factor  |
| $F_c$          | Calculated structure factor  |
| $T$            | Total diffractometer count during the measurement of a reflection  |
| $b$            | total background count during the measurement of a reflection  |
| $I$            | Intensity of the reflection, $I=T-b$   |
| $\sigma(I)$    | Estimated standard deviation (e.s.d.) for the intensity of a reflection where<br>$\sigma(I) = (T + (kI)^2 + b)^{\frac{1}{2}}$ and $k=0.04$ for all structures in this thesis (1) |
| $w$            | The weight associated with each reflection. Observation weights (1) are obtained from  |



the expression

$$w^{\frac{1}{2}} = 2 |F_o| / (T + (kI)^2 + b)^{\frac{1}{2}}$$

|                                  |   |
|----------------------------------|---|
| $E_o$                            | Observed normalized structure factor  |
| $E_c$                            | Calculated normalized structure factor  |
| $E_h$                            | Normalized structure factor corresponding to the reflection with indices $h=(h_1 \ h_2 \ h_3)$  |
| $\phi_w$                         | Phase angle of the reflection with indices $h=(h_1 \ h_2 \ h_3)$  |
| $R$                              | Conventional residual<br>$R = \sum   F_o  -  F_c   / \sum  F_o $  |
| $R_w$                            | Weighted residual (2)<br>$R_w = \{ \sum w    F_o  -  F_c   ^2 / \sum w  F_o ^2 \}^{\frac{1}{2}}$  |
| Sigma-2 equation (4)             | $\phi_h \simeq < \phi_{h-k} + \phi_k >_k$   |
| Tangent formula (5)              | $\tan \phi_w = \frac{\sum_k  E_{h-k} E_k  \sin(\phi_{h-k} + \phi_k)}{\sum_k  E_{h-k} E_k  \cos(\phi_{h-k} + \phi_k)}$                   |
| Weighted mean                    | $l_{av} = \sum (l_i / \sigma_i^2) / \sum (1 / \sigma_i^2)$<br>where $\sigma_i$ is the e.s.d. associated with each observation $l_i$ (6) |
| Standard deviation from the mean | $\sigma(l_{av}) = [\sum (l_i - l_{av})^2 / n(n-1)]^{\frac{1}{2}}$<br>where $n$ is the number of observations being averaged             |





$f_X$ 

Atomic form factor of the atom X; when written as  $f_X(s)$ , this denotes the value of the atomic form factor as a function of  $s = \sin \theta / \lambda$  where  $\lambda$  is the radiation wavelength. Form factors for the neutral atoms S, Cl, O, C and N were derived from the coefficients published by Cromer and Mann (7) with the program Cromercurve (8). The form factor for hydrogen is from Mason and Robertson (9). Corrections for the anomalous dispersion of Cu K $\alpha$  X-rays by S and Cl are  $\Delta f'_S$ , 0.3;  $\Delta f'_{Cl}$ , 0.3;  $\Delta f''_S$ , 0.6;  $\Delta f''_{Cl}$ , 0.7 electrons (10)

The form of the temperature factor for atoms which are assigned isotropic thermal parameters is  $T = \exp[-8\pi^2 U_{iso} (\sin \theta / \lambda)^2]$  where  $U_{iso}$  is the mean square amplitude of vibration in  $\text{\AA}^2$ .

The form of the temperature factor for atoms which are assigned anisotropic thermal parameters is  $T = \exp[-2\pi^2 (U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{23}k\ell b^*c^* + 2U_{13}h\ell a^*c^* + 2U_{12}hka^*b^*)]$  where  $a^*$ ,  $b^*$  and  $c^*$  are the lengths of the reciprocal lattice unit cell edges and the coefficients  $U_{ij}$  are the mean square amplitudes of vibration in  $\text{\AA}^2$ . A more detailed explanation of crystallographic nomenclature and standard procedures can be found in (10) and (11).



## CHAPTER I

## THE CRYSTAL AND MOLECULAR STRUCTURE OF OUABAIN TETRAHYDRATE

1.1 INTRODUCTION

Following the discovery and isolation of the membrane bound  $\text{Na}^+$ ,  $\text{K}^+$  - dependent adenosine triphosphate phosphohydrolase,  $[\text{Na}^+, \text{K}^+]$ -ATPase, by Skou (12) in 1957 from the leg nerves of the shore crab Carcinus Maenus, many investigations have been performed on the inhibition of this enzyme by the cardiac glycosides, with renewed speculation about the way these phenomena are related to the cardiotonic properties of these drugs (13,14,15,16). Although X-ray crystallographic studies have been performed on several steroid components of the cardiac glycosides (17,18,19,20) no conjectures on the manner in which these compounds interact with  $[\text{Na}^+, \text{K}^+]$ -ATPase have been presented using the X-ray results. Prior to 1957, information about the structure-activity relationships for the various cardiac glycosides and their chemical modifications, had been obtained from a comparison of their lethal doses in animals (21).

Clinically the fundamental action of the cardiac glycosides is to augment the contractile state of the heart. This is often referred to as a positive inotropic effect. Pharmacologically, they act in the following three ways: (22)

- (i) They increase cardiac muscle contractility with overall improvement of cardiac function.
- (ii) They slow the heart-beat by prolonging the refractory period of the atrioventricular node and 'bundle of HIS'.



- (iii) They increase the sensitivity of the sinoatrial node to vagal control.

Historically, the first scientific identification and use of the cardiac glycosides dates back to 1785 and the work of Withering who identified the cardioactive principle digitalis in extracts of the purple foxglove and used it effectively in the treatment of dropsy (23). During the period between 1785 and 1920 a large number of cardioactive steroids containing one or more sugar residues were isolated and identified in members of the plant orders Apocynaceae, Lilliaceae, Moraceae, Scrophulariaceae and Ranunculaceae.

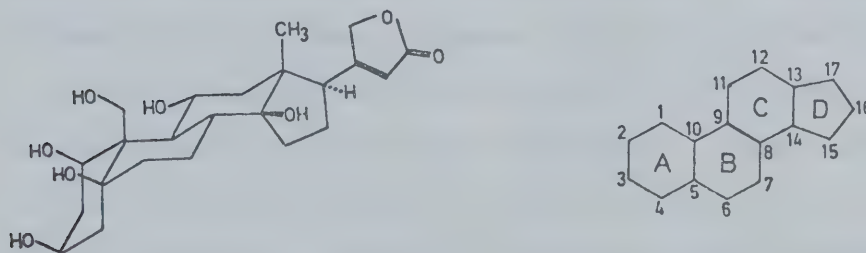


Figure 1.1 The structural formula of ouabagenin: 1 $\beta$ ,3 $\beta$ ,5,11 $\alpha$ ,14,19-hexahydroxy-5 $\beta$ -card 20(22)-enolide. The numbering scheme and ring designation for the cyclopentanoperhydrophenanthrene nucleus is shown to the right.

The cardiac glycoside ouabain was first isolated by Arnaud (24) in 1888 from the bark of the ouabaio tree. It was identified by Arnaud to be a rhamnoside but the chemical elucidation of the aglycone structure required considerable effort on the part of Jacobs and Bigelow, Mannich and Siewert, Sneed and Turner, Tamm and others. A summary of the work done in the determination of this structure and other members of





cardiac glycosides is given by Fieser and Fieser (21). The structural formula for the aglycone of ouabain (ouabagenin) is shown in Figure 1.1 together with the numbering scheme for the cyclopentanoperhydrophenanthrene nucleus.

#### 1.1.1 Structural properties of the cardiac glycosides

Enzymatic or acid hydrolysis of the cardiac glycosides shows that they are composed of two structurally distinct entities - a steroid referred to as the genin and one to four pyranose sugars. The difficulty in classification of the cardiac glycosides is in part due to the wide variety of sugar components. Fieser and Fieser (21) list 20 sugar components with their structural formulae.

The cyclopentanoperhydrophenanthrene nucleus is common to all steroid components of the cardiac glycosides. The stereochemistry of the ring junctions for the most potent drugs is A/B-cis, B/C-trans, and C/D-cis. A trans A/B ring fusion occurs in uzarigenin and cortoxigenin glycosides. However the cardiac activity of the former is considerably less than that observed in digitoxin and strophanthidin glycosides (21).

All cardiac glycosides contain an unsaturated 5- or 6- membered lactone ring at the 17 $\beta$ -position of the steroid nucleus. Cardioactive steroids containing a  $\gamma$ -butenolide substituent are generally found in plant tissues and are called cardenolides. The glycosides containing a 6-membered lactone ring are found mainly in animal tissues (toads) and these steroids are known as the bufanolides.

The unsaturated lactone ring and carbonyl moiety appear essential for inotropic activity (13). Saturation of the lactone



produces a non-planar ring and results in either a reduction of drug activity (ouabain, digoxin, digitoxin) or a complete loss of activity (strophanthidin). Hydrolysis of the lactone ring abolishes activity (13).

The  $3\beta$ -hydroxyl substituent, whether free or participating in a glycosidic linkage, is common to all members of the cardenolide series and is apparently essential for cardiac activity. Repke (13) has observed that detoxification of the cardenolides in the liver proceeds via the oxidation of the  $3\beta$ -hydroxyl to give a 3-keto derivative followed by its reduction to give a  $3-\alpha$ -hydroxyl substituent. It is believed that one of the actions of the sugar components is to retard the epimerization of the 3-O-substituent.

The 14-hydroxyl group is apparently important in the activity of both series in that all cardiac glycosides contain this substituent except for several bufagin derivatives which contain a  $14\beta$ ,  $15\beta$ -oxide ring. Introduction of a 8,14- or 14,15-double bond by dehydration of the 14-hydroxyl group abolishes drug activity (13,25). However this process also alters the cis C/D ring configuration. Curiously, however, it does not appreciably change the carbonyl-oxygen...3-hydroxyl separation which was found to be  $13.2 \overset{\circ}{\text{\AA}}$  in digitoxigenin (18) and  $13.4 \overset{\circ}{\text{\AA}}$  in  $\Delta$ -8,14-anhydrodigitoxigenin (19).

The presence or absence of free or esterified substituents at the 1,5,11,12,16 and 19-positions of the genin result in a variation in the toxicity and activity of the cardiac glycosides which is dependent on animal species (21). The 19-methyl substituent may occur as a primary alcohol (ouabain) or as an aldehyde (strophanthidin). These variations are associated with a modification of drug



activity which are an order of magnitude less than the changes observed for instance in the case where the 14-hydroxyl substituent is eliminated.

The introduction of a 16,17- or 5,6-double bond does not abolish inotropic activity (13,25). This indicates that the stereochemistry of the fused ring system has some degree of variability without loss of drug activity.

#### 1.1.2 Effects of the cardiac glycosides on $[\text{Na}^+, \text{K}^+]$ -ATPase

$[\text{Na}^+, \text{K}^+]$ -ATPase is important in maintaining intracellular levels of  $\text{K}^+$  and  $\text{Na}^+$  cations. This enzyme catalyses the hydrolysis of the terminal phosphate of ATP in the presence of an equimolar concentration of  $\text{Mg}^{++}$  but in addition requires  $\text{Na}^+$  and  $\text{K}^+$  for its activity (12). This enzyme is associated with the cell membrane and efforts to separate it from the membrane result in at least partial inactivation. Treatment of the enzyme preparations with bile salts or detergents disrupts the membrane resulting in inactivation of the enzyme (15).

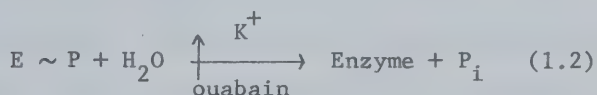
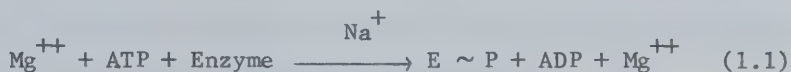
The relationship of  $[\text{Na}^+, \text{K}^+]$ -ATPase with the cardiac glycoside activity is highly significant in that all potent cardiotonic steroids are inhibitors of this enzyme (15). Similarly, compounds which resemble the cardiac glycosides but which are incapable of a positive inotropic effect do not inhibit  $[\text{Na}^+, \text{K}^+]$ -ATPase.

The characterization of the number and kind of intermediates involved in the overall reaction is not yet definite due to the complexity of the enzyme system and the wide range of experimental condi-





itions in which investigations can be pursued. In particular these investigations tend to depend on the method of enzyme preparation, the tissue source, temperature and the concentration of uni- and divalent cations for both in vivo and in vitro conditions. The reaction is however generally represented as a two step process given by equations 1.1 and 1.2.



Several authors (15) have found that the formation of a phosphorylated intermediate is part of the reaction series and the  $\text{Na}^+$  is required for the formation of this intermediate. However recent work by Allen, Harris and Schwartz (26) and by Charnock, Cook and Opit (27) suggests that at least two reaction pathways may occur under different conditions of temperature and substrate and ion concentrations.  $\text{Na}^+$  may not be required in one of these pathways.

Digitalis-enzyme complexes have been observed by Schwartz (26, 28) using  $^3\text{H}$ - enriched ouabain under varying conditions of temperature and ion concentration. Two ionic conditions,  $\text{ATP} + \text{Mg}^{++} + \text{Na}^+$ , and  $\text{Mg}^{++} + \text{P}_i$ , stimulate maximal binding of ouabain to calf brain membranal  $[\text{Na}^+, \text{K}^+]\text{-ATPase}$ . Under these conditions, two drug-enzyme complexes are formed which, in the absence of  $\text{K}^+$ , show non-identical dissociation characteristics. The differences in stabilities of the complexes were attributed to multiple binding conformations of the enzyme and support the view that the enzyme represents an allosteric system.



Recent studies by Wilson, Hanna and Sevitz (29), on the inhibition of calf brain membranal  $[\text{Na}^+, \text{K}^+]\text{-ATPase}$  by cardioactive sterols, have indicated each molecule of enzyme to be inhibited by one molecule of sterol. The inhibition of the enzyme was shown to be reversible. The enzyme-ouabain dissociation constant is  $1 \times 10^{-6} \text{ M}$ . Inhibition studies in the presence of two cardioactive sterols using ouabain, digitoxigenin and gammabufatolin indicated that the three steroids compete for an identical binding site.

The concentration of inhibitor ( $I_{50}$ ) required for the 50% inhibition of this enzyme was determined for 27 cardiotoxic sterols and chemically related compounds (29). Both the free aglycones and the mono-, di- and trisaccharides of the cardioactive steroids combine with the enzyme. The  $I_{50}$  values for the digitoxigenin and digoxigenin digitoxosides range from 0.15 to 0.55  $\mu\text{M}$  and 0.50 to 1.10  $\mu\text{M}$  respectively. The most potent inhibitors are the mono-saccharides and the least potent are the free aglycones. The  $I_{50}$  values for ouabain and dihydroouabain are 1.00 and 6.60  $\mu\text{M}$  respectively. The  $I_{50}$  values of resibufogen (14 $\beta$ ,15 $\beta$ -oxide ring) and uzarigenin (trans A/B ring junction) are 4.50 and 2.40  $\mu\text{M}$  respectively.

Based on these studies, a model for the complementary binding site on the enzyme for the cardioactive sterols was proposed (29). In this model three loci of attachment were considered using digitoxigenin as a representative inhibitor. The three sites are

- (i) the 3 $\beta$ -hydroxyl substituent or one of the hydroxyls of the sugar residues attached by a glycosidic linkage to the 3 $\beta$ -position of the steroid;



- (ii) the 14-hydroxyl substituent;
- (iii) the lactone ring attached to the 17 $\beta$ -position of the steroid.

Repke (13) considered the lactone carbonyl to interact with the  $[\text{Na}^+, \text{K}^+]$ -ATPase by acting as a hydrogen bond acceptor. Wilson *et al* (29) suggest that a  $\pi$ -bonding interaction between the sterol lactone and the enzyme may be possible. The implication of the 14-hydroxyl as a binding site was strengthened by the 30-fold decrease in the inhibitory effectiveness of the  $\Delta$ -8,14 and  $\Delta$ -14,15-anhydrodigitoxigenin derivatives (13).

Interest in the crystal structure of ouabain was stimulated by Dr. J.S. Charnock in the Department of Pharmacology at this University. This study was considered a worthwhile endeavour for three reasons in addition to the role of ouabain as an inhibitor of  $[\text{Na}^+, \text{K}^+]$ -ATPase. They are:

- (i) No crystal structure determinations of intact cardiac glycosides have been performed to date.
- (ii) The question of whether ouabain is an  $\alpha$ - or  $\beta$ -rhamnoside has not definitely been answered. J.S. Mills (15,30) has interpreted the ouabain structure as a  $\beta$ -L-rhamnoside for reasons known only to him. Fieser and Fieser (21) maintain the validity of the Klyne rule (31) which predicts that all cardiac L-glycosides have an  $\alpha$ -glycosidic linkage and that all D-glycosides have a  $\beta$ -linkage.
- (iii) The presence of three axial oxygen atoms at the 1,3 and 5-positions of the A ring suggests that





some sort of stabilization interactions may occur in order for the overall conformation of ouabagenin to agree with that observed in digitoxigenin.

## 1.2 EXPERIMENTAL

Ouabain was obtained from the Sigma Chemical Company as the octahydrate, in the form of soft colorless tetragonal platelets. The crystals were extinguished at all positions of the stage of a petrographic microscope when viewed between crossed polarizing filters through the plate direction. The commercial sample was recrystallized by evaporating an aqueous solution. This yielded crystals showing optical and morphological properties similar to the Sigma sample.

Preliminary diffraction photographs indicated that the octahydrate was unsuitable for a crystal structure analysis due to the poor X-ray reflectivity of the crystals. Precession photographs of the  $h0l$  and  $0kl$  zones showed mm diffraction symmetry with the odd-ordered reflections of the three principal axes systematically unobserved. Photographs of the higher order layers were unsatisfactory probably due to a lack of crystalline order. Cell dimensions measured with a circular measuring screen were  $a=b=11.53(2)\text{\AA}$  and  $c=54.4(1)\text{\AA}$ . A density of  $1.32\text{ g/cm}^3$  was calculated corresponding to 8 formula units of  $\text{C}_{29}\text{H}_{44}\text{O}_{12}\cdot 8\text{H}_2\text{O}$  (molecular weight 728.8) in a unit cell volume of  $7232.0\text{\AA}^3$ . This agreed with the value of  $1.32(1)\text{ g/cm}^3$  obtained by the method of flotation, using a chlorobenzene-bromobenzene solution. All evidence suggested the space groups is  $P4_2 2_1 2$  although a definite identification was not possible due



to the incomplete film record of the diffraction pattern. This was unfortunate, as Trevedi (32) has suggested that octahydrate crystals are monoclinic and pseudo-tetragonal on the basis of refractive index measurements. In the search for a more suitable crystalline specimen a second hydrated modification was obtained by the vapor diffusion of diethyl ether into a 1% solution of the octahydrate in 70:25:5 methanol:ethanol:water solvent. The crystals exhibited a columnar habit with 222 morphology and a strong birefringence.

Table 1.1

Cell data and physical constants for ouabain tetrahydrate.

|                               |   |
|-------------------------------|---|
| Formula                       | $C_{29}H_{44}O_{12} \cdot 4H_2O$                                      |
| Molecular weight              | 656.7   |
| Crystal class                 | Orthorhombic 222  |
| Space group                   | $P2_1^2 2_1^2 2_1$  |
| Cell dimensions               | $a=12.101(1)\text{\AA}$<br>$b=28.092(3)$<br>$c=9.678(1)$              |
| Volume                        | $V=3290.3\text{\AA}^3$  |
| Density                       | observed $1.33(1) \text{ g/cm}^3$<br>calculated $1.33 \text{ g/cm}^3$ |
| Cell content                  | $z=4$ molecules/cell  |
| Linear absorption coefficient | $\mu=9.2 \text{ cm}^{-1}$ (Cu K $\alpha$ )                            |



Preliminary diffraction photographs of this modification indicated the crystals were suitable for a structure determination as the observed X-ray diffraction pattern extended to the limit of the Cu K $\alpha$  sphere. When left exposed to the air over a period of several days to several weeks, the crystals became internally lineated parallel to the columnar direction probably due to the absorption of water. Crystals grown in a larger percentage of ethanol or in the absence of water tended to be acicular in habit and more susceptible to this type of decomposition.

Unit cell data for this crystal modification are listed in Table 1.1.

Weissenberg photographs exhibited mm diffraction symmetry with the odd-ordered reflections of the three principal axes systematically unobserved. Thus the crystals are orthorhombic with space group  $P2_1^2 2_1^2 2_1^2$ . Cell dimensions and intensity data were obtained for a crystal  $0.3 \times 0.2 \times 0.5$  mm (a x b x c) mounted with the c-axis coincident with the diffractometer  $\emptyset$ -shaft. Unit cell parameters and their e.s.d.'s were obtained by a least-squares procedure (33) using the angular coordinates of 12 accurately centered reflections. The crystal density was determined by the method of flotation using a chlorobenzene-bromobenzene solution. A chemical analysis of the crystal was not performed so that the identity of the solvent of crystallization was not determined.

Reflection intensities were measured with the FACS-1 diffractometer using graphite monochromatized Cu K $\alpha$  radiation at a take-off angle of  $4.5^\circ$ . Reflections were scanned in the  $\theta/2\theta$  moving crystal/moving detector mode at a rate of  $2^\circ 2\theta/\text{min.}$  for a standard scan width of  $1.4^\circ 2\theta$ , modified for the dispersion of the  $\alpha_1\alpha_2$  doublet. Stationary 10





second backgrounds were counted at the  $2\theta$  boundaries of each scan. A total of 3192 unique reflections were measured in one octant of reciprocal space and  $3^\circ < 2\theta < 129.5^\circ$ . Crystal decomposition was monitored by the periodic measurement of 3 standard reflections. During the data collection, the crystal developed small internal lineations and a 1% average decrease in the intensities of the three standard reflections.

The intensity data were scaled and corrected for decomposition, Lorentz and polarization effects (34) using the N.R.C. programs (35). A total of 2850 reflections were found to have intensities  $I > 3\sigma(I)$  and were considered observed. An observational weight was calculated for each reflection.

To facilitate the use of direct methods in the solution of the structure, an auxilliary set of intensity data was collected by a similar procedure using graphite monochromatized Mo  $K\alpha$  radiation and a large crystal sealed in a glass capillary. A total of 3136 lattice points in one octant of reciprocal space with  $45^\circ < 2\theta < 60^\circ$  and  $19^\circ < 2\theta < 25^\circ$  were examined. The intensity data were corrected for decomposition, Lorentz and polarization effects.

The scale constant  $k'$  in the equation  $|F|_M^2 = k' |F|_C^2$  where the subscripts M and C refer to the Mo  $K\alpha$  and Cu  $K\alpha$  data respectively, was determined to be 0.3839 by a least-squares procedure. The quantity minimized was  $\sum (w_M |F|_M^2 - k' w_C |F|_C^2)^2$  where the summation was for all reflections common to both data sets. The weights  $w_M$  and  $w_C$  were obtained by the following equations:

$$\begin{aligned} \text{for } |F|_C^2 \leq 2500, \quad w_C &= (0.02)^2 |F|_C^2 \\ 2500 < |F|_C^2 < 28900, \quad w_C &= 1.0 \end{aligned}$$



$$|F|_C^2 \geq 28900 \quad , \quad w_C = (28900)^2 / |F|_C^2$$

$$\text{and for } |F|_M^2 \leq 900 \quad , \quad w_M = (0.033)^2 |F|_M^2$$

$$900 < |F|_M^2 < 14400 \quad , \quad w_M = 1.0$$

$$|F|_M^2 \geq 14400 \quad , \quad w_M = (14400)^2 / |F|_M^2$$

The number of unique reflections in the combined data set was 5390.

### 1.2.1 Solution and refinement of the structure

Normalized structure factors  $|E|$  were derived from the equation

$$|E| = \left\{ \frac{k(\sin^2 \theta)^{\frac{1}{2}}}{\sum_{hkl} \frac{1}{f^2}} \right\} \cdot |F|$$

where  $k(\sin^2 \theta)$  is a normalization function which places  $|F_{hkl}|$  onto absolute scale for atoms at rest. When  $\ln\{k(\sin^2 \theta)\}$  was obtained from the linear Wilson plot (36) shown in Figure 1.2, reflections with  $\sin^2 \theta > 0.2$  tended to have overestimated values of  $|E|$ . To produce a distribution of large  $|E|$  which was independent of  $\sin^2 \theta$  the function  $\ln\{k(\sin^2 \theta)\}$  was obtained from the non-linear Wilson plot shown in Figure 1.2.

The E-statistics are given in Table 1.2 with the theoretical values for centric and acentric space groups (37). A list of reflections with  $|E| > 2.0$  sorted into the eight parity groups is listed in Table A.2 of the Appendix.



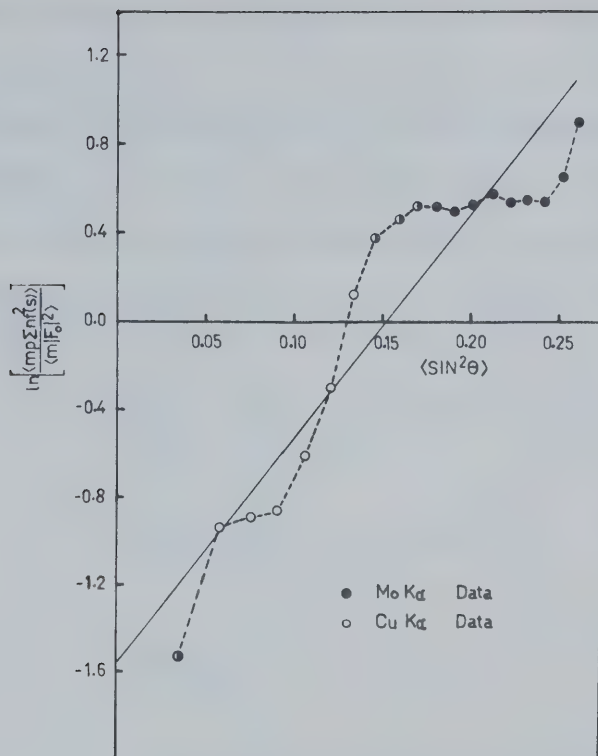


Figure 1.2. Wilson plot for ouabain based on the combined data set. The non-linear Wilson plot is indicated by the dashed line,  $m$  is the reflection multiplicity,  $p$  is a factor to account for systematically absent reflections.

Table 1.2

E-statistics and distribution for ouabain tetrahydrate

|                             | This structure | Acentric | Centric |
|-----------------------------|----------------|----------|---------|
| $\langle  E  \rangle$       | 0.879          | 0.866    | 0.798   |
| $\langle  E ^2 \rangle$     | 0.987          | 1.000    | 1.000   |
| $\langle  E ^2 - 1 \rangle$ | 0.759          | 0.736    | 0.968   |
| $ E  > 3.0$                 | 0.09%          | 0.01%    | 0.27%   |
| $ E  > 2.5$                 | 0.50%          | 0.19%    | 1.24%   |
| $ E  > 2.0$                 | 2.41%          | 1.83%    | 4.55%   |



The sigma-1 formulae given by equations 1.2, 1.3 and 1.4 were evaluated for all two-dimensional reflections with  $|E| > 2.0$  belonging to the (eee) parity group. From this evaluation the phases  $\emptyset$  (12 0 6) and  $\emptyset$  (12 22 0) had indications of  $\pi$  and 0 radians respectively. The probabilities  $P_+$  that the phases were correctly determined were evaluated by equation 1.5 to be 0.97 and 0.93 for (12 0 6) and (12 22 0) respectively.

$$S(E_{2h,2k,0}) \approx \sum_{\ell} (|E_{h k \ell}|^2 - 1) (-1)^{\ell+h} \quad (1.2)$$

$$S(E_{2h,0,2\ell}) \approx \sum_k (|E_{h k \ell}|^2 - 1) (-1)^{k+\ell} \quad (1.3)$$

$$S(E_{0,2k,2\ell}) \approx \sum_h (|E_{h k \ell}|^2 - 1) (-1)^{h+k} \quad (1.4)$$

$$P_+(E_{2h}) = 0.5 \left( 1 + \tanh \frac{\sigma_3 \sigma_2^{-3/2}}{2} |E_{2h}| |\Sigma_1| \right) \quad (1.5)$$

where  $\Sigma_1$  is the accumulated total corresponding to the right hand side of equations 1.2, 1.3 and 1.4. The product  $\sigma_3 \sigma_2^{-3/2}$  in equation 1.5 has a value of 0.0743.

A detailed description of the selection of origin and enantiomorph defining reflections plus the manual evaluation of new reflection phases using the sigma-2 formula is given in the Appendix. The starting set of the reflection phases which were used to determine the correct structure are listed in Table 1.3.





Table 1.3

The starting set of reflection phases used to initiate the phase generation and tangent refinement process.

| reflection |    |    | $ E $ | $\phi$ (radians) |  |
|------------|----|----|-------|------------------|--|
| 0          | 2  | 11 | 2.79  | 0                | origin defining reflections  |
| 1          | 0  | 12 | 2.88  | 0                |  |
| 4          | 9  | 0  | 2.05  | 0                |  |
| 10         | 0  | 7  | 3.55  | $\pi/2$          | enantiomorph*  |
| 12         | 0  | 6  | 2.49  | $\pi$            | evaluated from sigma-1   |
| 12         | 22 | 0  | 3.52  | 0                |  |
| 6          | 16 | 3  | 3.11  | 0                | evaluated from hand phasing and constrained to be constant for one cycle of phase refinement |
| 1          | 2  | 1  | 2.22  | $7\pi/8$         |  |
| 10         | 2  | 4  | 2.25  | $3\pi/8$         |  |

\* The value of  $\phi$  (10 0 7) which resulted in the correct optic hand was  $-\pi/2$ .



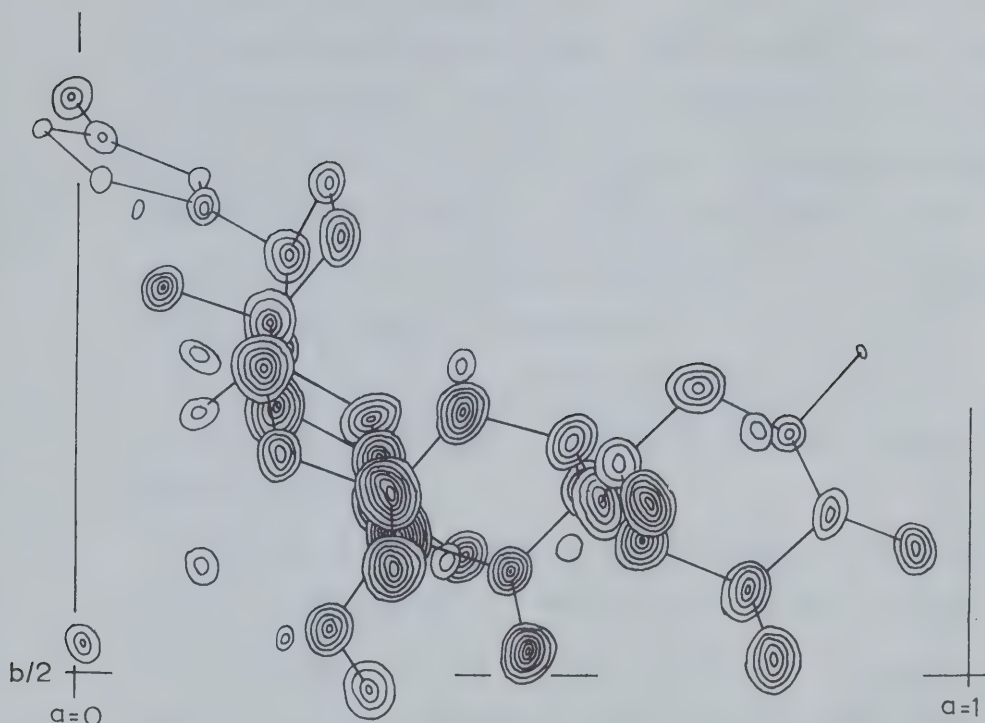


Figure 1.3 The E-map showing the positions of the carbon and oxygen atoms of the ouabain molecule. All maxima above a threshold of 0.7 are contoured in intervals of 0.4.

The phases of 317 of a possible 319 reflections with  $|E| > 1.71$  were determined by an iterative application of the tangent formula (5) using the phase refinement program of Huber and Brisse (35). The 41 non-hydrogen atoms of the ouabain molecule were readily identified from an E-Fourier synthesis based on this phase information. This E-map is depicted in Figure 1.3. The atoms, which were later found to have a large thermal motion, appeared as the least prominent peaks in this map. The locations of the solvent molecules of crystallization could not be determined.



The value of the conventional residual  $R$  for the structure factor calculation corresponding to the positional parameters of the 41 atoms located from the E-map, using a scale of 0.4613 and an overall temperature factor  $B=2.57\text{\AA}^2$  (determined by the Wilson plot method (36)) was 0.32. The positional and thermal parameters of the non-hydrogen atoms were refined by the method of least-squares using the block-diagonal approximation with the program NRC-10(35). The quantity minimized was  $D = \sum w (|F_o| - |F_c|)^2 = \sum w \Delta^2$ . Each reflection was assigned a weight  $w=1.0$  until the late stages of the refinement. None of the hydrogen atom parameters were refined.

A difference Fourier computed at the residual 0.192 showed 4 diffuse maxima located in a channel between symmetry related ouabain molecules. These peaks were identified as the oxygen atoms of four water molecules of crystallization. Each atom was assigned an isotropic thermal parameter  $B=4.0\text{\AA}^2$ . The separation of the 'water' oxygen atoms from each other suggested the four atoms to be held together by a series of hydrogen bonds.

After several least-squares cycles in which the positional and thermal parameters of all 45 atoms were refined, that data measured with Mo  $K\alpha$  radiation were discarded. This action was taken in order to avoid the effects of serious scaling errors between the two sets of intensity data. The refined values of thermal parameters for the solvent atoms ( $B=8.4$  to  $13.2\text{\AA}^2$ ) suggested the solvent of crystallization to be disordered.

The refinement was continued using the parameters corresponding to the residual 0.156 but allowing the occupational factors





of the solvent atoms to refine as well. The locations of the methylene and methine hydrogen atoms were calculated by the procedure of Cradwick (38) assuming a carbon-hydrogen separation of  $1.02\text{\AA}$ . The non-hydrogen atoms of the ouabain molecule were assigned anisotropic thermal parameters at the residual 0.131 and refined as such. The thermal parameters of the hydrogen atoms were set equal to the final isotropic thermal parameters of the bonded carbon atoms.

Four cycles of least-squares decreased the residual to 0.088. During this process the occupational factors for three of the solvent atoms decreased to values less than unity. The thermal parameters of the two inner members of the chain (O(W1) and O(S2)) were  $B=14.4$  and  $18.9\text{\AA}^2$ .

The locations of the remaining hydrogen atoms of the ouabain molecule were determined from a difference Fourier synthesis. The two sets of methyl hydrogen atoms were ordered. The positions of the hydroxyl hydrogen atoms indicated a complex network of hydrogen bonds. Several maxima and minima in the vicinity of the 'water' oxygen atoms suggested an anisotropic thermal description was warranted. Several large peaks were found midway between the last two members of the chain of solvent atoms (O(S1) and O(S2)).

The thermal parameters of the newly found hydrogen atoms were set equal to the final isotropic thermal parameters of the bonded carbon or oxygen atom. Several cycles of refinement using an anisotropic thermal model for the solvent atoms led to an unsatisfactory description for the atoms O(S1) and O(S2). The difference Fourier was then reexamined and the same two atoms



were interpreted to be the oxygen atoms of two disordered methanol molecules each having an occupational factor of 0.5. The two peaks midway between these atoms were interpreted as carbon atoms each having an occupational factor of 0.5. Each of these four atoms was given an isotropic thermal parameter  $B=10.0\text{\AA}^2$  and refined as such. The occupational factors of the two remaining solvent atoms ('water') were set to 1.0.

After nine cycles of least-squares using observational weights, all parameter shifts (except for (O(W1))) were less than 0.2 of the corresponding e.s.d. During this process, a number of reflections with large  $|F_o|$  and  $|F_c|/|F_o| > 1.15$  were suspected to have underestimated values of  $|F_o|$  due to counter paralysis and/or secondary extinction errors. These reflections were assigned a modified weight  $w'$  such that  $w'(|F_o| - |F_c|)^2 = 0.5$ . Convergence of the refinement process was achieved by applying the following series of relaxation factors to the calculated parameter shifts - 0.7, 0.9, 0.6, 0.5, 0.3, 0.5, 0.3 and 1.0.

The final values of the weighted and unweighted residuals,  $R_w$  and  $R$  respectively, were 0.082 and 0.061. The scale factor which places  $|F_o|$  on absolute scale was 0.2125.

A difference Fourier computed with the final calculated structure factors showed several large maxima with values between 0.60 and  $0.35\text{ e/\AA}^3$  in the vicinity of O(W1), O(W2) and O(S2). This indicated that the final set of refined parameters did not fully account for the disordering of the solvent. However it was decided that the residual disordering would not justify the expenditure of additional computing money and the refinement was terminated.



The final atomic parameters are listed in Table 1.4, 1.5 and 1.6. Estimated standard deviations are listed in parentheses and refer to the least significant digit. The observed and calculated structure factors are listed in Table 1.7 on a scale of 10X their absolute value.



Table 1.4

Positional parameters ( $\times 10^4$ ) for the non-hydrogen atoms of ouabain tetrahydrate.

| Atom  | x/a     | y/b     | z/c     |
|-------|---------|---------|---------|
| C(1)  | 5146(3) | 5498(1) | 3371(4) |
| C(2)  | 4343(3) | 5899(1) | 3057(4) |
| C(3)  | 4560(3) | 6145(1) | 1688(4) |
| C(4)  | 5772(3) | 6270(1) | 1545(4) |
| C(5)  | 6592(3) | 5870(1) | 1851(4) |
| C(6)  | 7771(3) | 6066(1) | 1719(4) |
| C(7)  | 8001(3) | 6457(1) | 2771(4) |
| C(8)  | 7788(3) | 6288(1) | 4237(4) |
| C(9)  | 6657(3) | 6026(1) | 4463(4) |
| C(10) | 6390(3) | 5644(1) | 3309(4) |
| C(11) | 6721(3) | 5837(1) | 5967(4) |
| C(12) | 6784(4) | 6251(1) | 6982(4) |
| C(13) | 7835(3) | 6550(1) | 6811(4) |
| C(14) | 7925(3) | 6700(1) | 5272(4) |
| C(15) | 7088(3) | 7108(1) | 5133(4) |
| C(16) | 7177(4) | 7384(2) | 6496(5) |
| C(17) | 7640(3) | 7034(1) | 7587(5) |
| C(18) | 8820(4) | 6266(2) | 7346(5) |
| C(19) | 7117(3) | 5197(1) | 3497(5) |
| C(20) | 8618(4) | 7253(1) | 8305(5) |
| C(21) | 9667(4) | 7422(2) | 7623(5) |
| C(22) | 8682(4) | 7362(2) | 9663(5) |
| C(23) | 9723(4) | 7602(1) | 9908(6) |
| O(1)  | 5003(2) | 5106(1) | 2416(3) |

/Contd.





Table 1.4 Continued.

| Atom  | x/a      | y/b      | z/c      | U <sub>iso</sub> |
|-------|----------|----------|----------|------------------|
| O(3)  | 4173(2)  | 5825(1)  | 619(3)   |                  |
| O(5)  | 6511(2)  | 5518(1)  | 775(3)   |                  |
| O(11) | 5790(2)  | 5557(1)  | 6385(3)  |                  |
| O(14) | 9030(2)  | 6891(1)  | 5111(3)  |                  |
| O(19) | 6688(2)  | 4909(1)  | 4598(3)  |                  |
| O(21) | 10305(3) | 7634(1)  | 8730(4)  |                  |
| O(23) | 10106(4) | 7763(1)  | 10975(4) |                  |
| C(1') | 3969(3)  | 6046(2)  | -661(4)  |                  |
| C(2') | 3684(3)  | 5637(2)  | -1637(4) |                  |
| C(3') | 2556(3)  | 5427(1)  | -1300(5) |                  |
| C(4') | 1677(4)  | 5811(2)  | -1133(5) |                  |
| C(5') | 2083(4)  | 6190(2)  | -92(6)   |                  |
| C(6') | 1286(5)  | 6606(2)  | 42(10)   |                  |
| O(1') | 3115(3)  | 6384(1)  | -563(3)  |                  |
| O(2') | 3663(3)  | 5811(1)  | -3022(3) |                  |
| O(3') | 2226(2)  | 5106(1)  | -2359(4) |                  |
| O(4') | 688(3)   | 5619(1)  | -546(4)  |                  |
| O(W1) | 7440(23) | 8375(4)  | 4163(16) |                  |
| O(W2) | 9154(5)  | 7722(2)  | 3610(7)  |                  |
| O(S1) | 5895(9)  | 9577(4)  | 7815(11) | 1152(32)         |
| O(S2) | 5981(13) | 8691(6)  | 5643(17) | 1777(59)         |
| C(S1) | 5723(23) | 9204(10) | 6548(26) | 1773(88)         |
| C(S2) | 5935(18) | 9234(8)  | 5445(23) | 1454(69)         |



Table 1.5

Anisotropic thermal parameters ( $\times 10^4$ )  
used to describe the atoms of Table 1.4.

| Atom  | $U_{11}^*$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{23}$ | $U_{13}$ |
|-------|------------|----------|----------|----------|----------|----------|
| C(1)  | 171(8)     | 174(8)   | 154(8)   | -15(7)   | -4(7)    | -7(7)    |
| C(2)  | 147(8)     | 216(9)   | 176(8)   | 6(7)     | -9(8)    | -9(7)    |
| C(3)  | 177(9)     | 181(8)   | 183(9)   | 10(7)    | -15(7)   | -43(8)   |
| C(4)  | 190(8)     | 162(7)   | 143(8)   | -5(7)    | 0(7)     | -27(7)   |
| C(5)  | 152(7)     | 169(7)   | 149(8)   | -2(7)    | -17(7)   | -1(7)    |
| C(6)  | 162(8)     | 234(9)   | 154(8)   | -15(7)   | -1(8)    | 24(8)    |
| C(7)  | 172(8)     | 237(9)   | 155(9)   | -37(8)   | 3(8)     | 6(7)     |
| C(8)  | 141(7)     | 194(8)   | 151(8)   | -10(7)   | 21(7)    | 1(7)     |
| C(9)  | 134(7)     | 187(8)   | 132(7)   | -11(6)   | 4(7)     | 1(7)     |
| C(10) | 140(8)     | 160(7)   | 160(8)   | 1(6)     | 8(7)     | -20(7)   |
| C(11) | 172(8)     | 198(8)   | 160(8)   | -30(7)   | 8(7)     | -5(7)    |
| C(12) | 203(9)     | 244(10)  | 139(8)   | -42(8)   | 0(8)     | -13(8)   |
| C(13) | 175(8)     | 231(9)   | 145(8)   | -20(8)   | -8(8)    | -21(8)   |
| C(14) | 148(7)     | 193(8)   | 172(9)   | -4(7)    | 2(7)     | -14(7)   |
| C(15) | 226(9)     | 191(9)   | 185(9)   | -5(8)    | -4(8)    | -39(9)   |
| C(16) | 245(11)    | 255(10)  | 265(12)  | 45(10)   | -88(10)  | -44(10)  |
| C(17) | 199(9)     | 249(10)  | 184(9)   | -7(8)    | -53(9)   | -8(8)    |
| C(18) | 215(10)    | 246(10)  | 210(10)  | 0(9)     | -17(9)   | -54(9)   |
| C(19) | 175(8)     | 175(8)   | 214(10)  | 7(7)     | 4(8)     | -10(8)   |
| C(20) | 232(10)    | 188(9)   | 218(10)  | -12(8)   | -15(8)   | -32(9)   |

\* These coefficients are defined in the Preface.

/Contd.



Table 1.5 Continued.

| Atom  | $U_{11}^*$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{23}$ | $U_{13}$ |
|-------|------------|----------|----------|----------|----------|----------|
| C(21) | 279(13)    | 269(11)  | 240(11)  | -93(10)  | -7(10)   | -16(11)  |
| C(22) | 295(12)    | 216(9)   | 196(10)  | 1(9)     | -19(9)   | -41(10)  |
| C(23) | 308(13)    | 179(9)   | 286(12)  | 15(9)    | -40(10)  | -114(11) |
| O(1)  | 170(5)     | 184(6)   | 198(6)   | -30(5)   | -23(5)   | -32(5)   |
| O(3)  | 189(6)     | 203(6)   | 176(6)   | 5(5)     | -4(5)    | -57(5)   |
| O(5)  | 190(6)     | 203(6)   | 159(6)   | -7(5)    | -40(5)   | 7(5)     |
| O(11) | 249(7)     | 261(7)   | 181(6)   | -96(6)   | 17(6)    | 9(6)     |
| O(14) | 186(6)     | 235(7)   | 195(7)   | -46(5)   | 2(6)     | 5(6)     |
| O(19) | 223(6)     | 217(6)   | 247(7)   | 30(6)    | 65(6)    | -21(6)   |
| O(21) | 275(8)     | 239(7)   | 315(10)  | -64(7)   | -19(7)   | -77(8)   |
| O(23) | 405(11)    | 327(10)  | 345(11)  | -14(9)   | -98(9)   | -155(10) |
| C(1') | 214(9)     | 237(9)   | 176(9)   | -26(8)   | 29(8)    | -67(8)   |
| C(2') | 181(9)     | 277(11)  | 185(9)   | -5(8)    | -4(9)    | -34(8)   |
| C(3') | 187(9)     | 229(10)  | 213(10)  | -17(8)   | -38(9)   | -7(8)    |
| C(4') | 175(9)     | 287(11)  | 305(13)  | 22(9)    | -67(11)  | -54(10)  |
| C(5') | 213(10)    | 247(10)  | 341(14)  | 52(9)    | -86(11)  | -111(11) |
| C(6') | 348(16)    | 360(17)  | 795(36)  | 150(15)  | -225(21) | -266(22) |
| O(1') | 266(8)     | 207(6)   | 301(9)   | 10(6)    | 0(7)     | -145(7)  |
| O(2') | 273(9)     | 432(10)  | 176(7)   | -60(8)   | 40(8)    | -35(7)   |
| O(3') | 223(8)     | 316(9)   | 294(9)   | -61(7)   | -109(8)  | -2(7)    |
| O(4') | 203(7)     | 388(10)  | 410(12)  | 0(8)     | -147(10) | 5(9)     |
| O(W1) | 3126(150)  | 935(42)  | 1409(62) | -743(73) | 689(47)  | -512(87) |
| O(W2) | 540(17)    | 616(19)  | 636(22)  | -50(16)  | 282(18)  | 149(18)  |

\* These coefficients are defined in the Preface.



Table 1.6

The positions and thermal parameters ( $\times 10^3$ ) for the hydrogen atoms of the ouabain molecule. The hydrogen atoms are numbered by multiplying the number of the bonded atom by 10 and adding, 1, 2 or 3.

| Atom   | x/a | y/b | z/c | U <sub>iso</sub> | Atom   | x/a  | y/b | z/c  | U <sub>iso</sub> |
|--------|-----|-----|-----|------------------|--------|------|-----|------|------------------|
| H(11)  | 498 | 537 | 436 | 35               | H(183) | 887  | 598 | 690  | 44               |
| H(21)  | 355 | 576 | 304 | 37               | H(191) | 712  | 500 | 258  | 39               |
| H(22)  | 440 | 615 | 384 | 37               | H(192) | 792  | 530 | 373  | 39               |
| H(31)  | 410 | 646 | 165 | 34               | H(211) | 1009 | 714 | 718  | 57               |
| H(41)  | 590 | 638 | 54  | 30               | H(212) | 949  | 767 | 686  | 57               |
| H(61)  | 833 | 579 | 187 | 37               | H(221) | 809  | 729 | 1040 | 51               |
| H(62)  | 788 | 620 | 73  | 37               | H(01)  | 428  | 508 | 225  | 37               |
| H(71)  | 882 | 656 | 268 | 38               | H(05)  | 593  | 533 | 85   | 39               |
| H(72)  | 750 | 675 | 256 | 38               | H(011) | 588  | 526 | 604  | 47               |
| H(81)  | 840 | 604 | 447 | 30               | H(014) | 905  | 711 | 460  | 44               |
| H(91)  | 604 | 628 | 443 | 30               | H(019) | 733  | 478 | 492  | 47               |
| H(111) | 743 | 563 | 607 | 36               | H(11') | 462  | 622 | -112 | 45               |
| H(121) | 610 | 647 | 683 | 41               | H(21') | 428  | 537 | -155 | 41               |
| H(122) | 676 | 611 | 798 | 41               | H(31') | 262  | 524 | -37  | 44               |
| H(151) | 729 | 733 | 430 | 44               | H(41') | 151  | 597 | -208 | 55               |
| H(152) | 629 | 698 | 500 | 44               | H(51') | 219  | 603 | 87   | 59               |
| H(161) | 771 | 767 | 638 | 51               | H(61') | 75   | 649 | 21   | 88               |
| H(162) | 640 | 751 | 680 | 51               | H(62') | 175  | 683 | 63   | 88               |
| H(171) | 703 | 698 | 832 | 39               | H(63') | 132  | 675 | -70  | 88               |
| H(181) | 875 | 623 | 840 | 44               | H(02') | 437  | 574 | -343 | 63               |
| H(182) | 942 | 637 | 723 | 44               | H(03') | 280  | 502 | -291 | 61               |
|        |     |     |     |                  | H(04') | 85   | 545 | -11  | 66               |







Table 1.7

The observed and calculated structure factor amplitudes, on the absolute scale  $\times 10$ , and the phase angles for ouabain tetrahydrate. The unobserved reflections are indicated by an asterisk (\*). Reflections which were given a modified weight during the terminal stages of the refinement are indicated by a dagger (†).

[illegible]



[illegible]

Continued...





[illegible]





| L               | PO | PC | ALPHA | L               | PO | PC | ALPHA | L               | PO | PC | ALPHA | L               | PO | PC | ALPHA | L               | PO | PC | ALPHA | L               | PO | PC | ALPHA          | L               | PO | PC | ALPHA | L              | PO | PC | ALPHA |
|-----------------|----|----|-------|-----------------|----|----|-------|-----------------|----|----|-------|-----------------|----|----|-------|-----------------|----|----|-------|-----------------|----|----|----------------|-----------------|----|----|-------|----------------|----|----|-------|
| N= 10, K= 14    |    |    |       | 1 234 20 165.4  |    |    |       | N= 11, K= 5     |    |    |       | 3 50 52 220.5   |    |    |       | 0 65 56 276.0   |    |    |       | 0 84 88 189.0   |    |    |                | N= 12, K= 14    |    |    |       | 0 52 55 270.0  |    |    |       |
| 1 81 76 236.4   |    |    |       | N= 10, K= 22    |    |    |       | 0 130 136 90.0  |    |    |       | 1 59 52 251.9   |    |    |       | 1 70 62 183.0   |    |    |       | 1 274 21 77.6   |    |    |                | 1 274 21 77.6   |    |    |       | 1 254 20 75.3  |    |    |       |
| 1 85 45 224.4   |    |    |       | 0 244 10 0.0    |    |    |       | 1 44 53 331.3   |    |    |       | 5 65 65 316.3   |    |    |       | 2 54 57 243.0   |    |    |       | 2 104 110 131.9 |    |    |                | 0 39 40 0.0     |    |    |       | 2 254 20 75.3  |    |    |       |
| 4 40 65 58.1    |    |    |       | 2 113 110 115.4 |    |    |       | 3 37 38 87.6    |    |    |       | N= 11, K= 12    |    |    |       | N= 11, K= 20    |    |    |       | 3 87 80 39.7    |    |    |                | 1 82 74 205.2   |    |    |       | 2 40 31 126.3  |    |    |       |
| 5 62 61 156.8   |    |    |       | 4 114 112 196.8 |    |    |       | 5 122 121 74.5  |    |    |       | 0 244 21 270.0  |    |    |       | 0 24 10 270.0   |    |    |       | 4 63 67 351.5   |    |    |                | 2 47 45 277.4   |    |    |       | 3 39 39 159.1  |    |    |       |
| 6 94 78 139.5   |    |    |       | 6 97 92 75.8    |    |    |       | 6 97 92 75.8    |    |    |       | 1 82 86 234.7   |    |    |       | 1 47 62 186.3   |    |    |       | 5 35 42 156.8   |    |    |                | 3 58 49 139.1   |    |    |       | 4 224 9 12.4   |    |    |       |
| N= 10, K= 15    |    |    |       | N= 10, K= 23    |    |    |       | N= 11, K= 6     |    |    |       | 2 116 117 58.9  |    |    |       | N= 12, K= 7     |    |    |       | 0 85 86 180.0   |    |    |                | N= 12, K= 15    |    |    |       | N= 13, K= 6    |    |    |       |
| 0 157 158 0.0   |    |    |       | 0 99 93 0.0     |    |    |       | 0 183 178 50.0  |    |    |       | 3 29 38 89.8    |    |    |       | 0 12, K= 0      |    |    |       | 1 130 128 292.9 |    |    |                | 0 88 85 0.0     |    |    |       | 0 48 49 270.0  |    |    |       |
| 1 74 85 302.3   |    |    |       | 1 224 3 174.9   |    |    |       | 1 284 41 7.8    |    |    |       | 4 56 44 248.5   |    |    |       | 0 39 35 0.0     |    |    |       | 2 35 33 153.4   |    |    |                | 1 124 131 250.2 |    |    |       | 1 55 56 275.8  |    |    |       |
| 2 49 65 164.4   |    |    |       | N= 11, K= 0     |    |    |       | 2 120 143 53.5  |    |    |       | 5 53 56 299.6   |    |    |       | 1 76 73 270.0   |    |    |       | 3 45 33 316.3   |    |    |                | 2 116 118 298.6 |    |    |       | 2 39 46 240.4  |    |    |       |
| 3 73 67 154.9   |    |    |       | 1 119 125 90.0  |    |    |       | 3 46 54 185.4   |    |    |       | N= 11, K= 13    |    |    |       | 3 77 70 0.0     |    |    |       | 3 32 32 270.0   |    |    |                | N= 12, K= 16    |    |    |       | N= 13, K= 7    |    |    |       |
| 4 51 56 139.0   |    |    |       | 2 182 178 180.0 |    |    |       | 4 274 25 225.5  |    |    |       | 0 85 87 90.0    |    |    |       | 4 111 107 180.0 |    |    |       | 5 83 88 325.8   |    |    |                | 0 234 29 180.0  |    |    |       | 0 55 47 270.0  |    |    |       |
| 4 50 50 112.4   |    |    |       | 3 55 58 270.0   |    |    |       | 5 28 27 253.9   |    |    |       | 1 48 37 246.1   |    |    |       | 5 83 80 90.0    |    |    |       | 1 274 33 285.6  |    |    |                | 1 31 34 215.7   |    |    |       | 1 41 46 71.4   |    |    |       |
| N= 10, K= 16    |    |    |       | 4 117 110 0.0   |    |    |       | 6 69 60 31.0    |    |    |       | 2 274 10 215.3  |    |    |       | N= 12, K= 1     |    |    |       | 0 150 162 180.0 |    |    |                | 2 224 29 268.7  |    |    |       | 2 59 59 109.1  |    |    |       |
| 0 284 24 180.0  |    |    |       | 6 112 120 0.0   |    |    |       | N= 11, K= 7     |    |    |       | 3 82 86 209.6   |    |    |       | 1 274 33 285.6  |    |    |       | 2 39 15 193.7   |    |    |                | N= 12, K= 17    |    |    |       | 3 49 60 165.3  |    |    |       |
| 1 115 125 196.7 |    |    |       | 7 224 7 270.0   |    |    |       | 0 50 52 270.0   |    |    |       | 4 93 84 175.4   |    |    |       | 1 284 11 323.1  |    |    |       | 3 46 54 316.7   |    |    |                | 0 108 98 180.0  |    |    |       | N= 13, K= 8    |    |    |       |
| 2 42 45 81.5    |    |    |       | N= 11, K= 1     |    |    |       | 1 46 63 226.4   |    |    |       | 5 234 17 27.3   |    |    |       | 2 103 110 202.5 |    |    |       | 4 63 66 59.4    |    |    |                | 1 77 81 322.4   |    |    |       | 0 254 22 270.0 |    |    |       |
| 3 63 67 202.8   |    |    |       | 0 49 48 90.0    |    |    |       | 2 163 185 242.7 |    |    |       | N= 11, K= 14    |    |    |       | 3 45 54 51.5    |    |    |       | 5 40 33 317.0   |    |    |                | N= 13, K= 0     |    |    |       | 1 110 109 22.3 |    |    |       |
| 4 56 60 5.3     |    |    |       | 1 32 25 180.7   |    |    |       | 3 95 95 97.4    |    |    |       | 0 76 69 270.0   |    |    |       | 4 66 79 441.5   |    |    |       | 5 83 89 12.9    |    |    |                | N= 13, K= 1     |    |    |       | 2 55 47 63.5   |    |    |       |
| 5 71 75 164.1   |    |    |       | 2 46 48 200.8   |    |    |       | 4 20 27 212.8   |    |    |       | N= 12, K= 2     |    |    |       | 1 86 79 441.5   |    |    |       | 0 274 21 180.0  |    |    |                | 1 128 134 270.0 |    |    |       | 3 48 44 101.5  |    |    |       |
| N= 10, K= 17    |    |    |       | 3 54 55 76.7    |    |    |       | 5 91 88 145.4   |    |    |       | 2 41 51 217.4   |    |    |       | 0 255 259 0.0   |    |    |       | 1 81 82 0.9     |    |    |                | 2 60 59 0.0     |    |    |       | N= 13, K= 9    |    |    |       |
| 0 274 25 0.0    |    |    |       | 5 87 90 16.2    |    |    |       | 6 37 46 267.7   |    |    |       | N= 11, K= 8     |    |    |       | 1 100 100 260.7 |    |    |       | 2 109 96 19.8   |    |    |                | 3 244 6 90.0    |    |    |       | 0 47 46 90.0   |    |    |       |
| 1 274 1 3.0     |    |    |       | 6 35 35 31.8    |    |    |       | 0 284 18 270.0  |    |    |       | 4 75 73 237.5   |    |    |       | 2 74 64 350.8   |    |    |       | 3 62 53 81.8    |    |    |                | 4 49 43 160.0   |    |    |       | 1 244 20 157.9 |    |    |       |
| 2 30 23 320.1   |    |    |       | N= 11, K= 2     |    |    |       | 1 106 113 150.4 |    |    |       | N= 11, K= 15    |    |    |       | 4 36 42 331.1   |    |    |       | 5 44 41 84.5    |    |    |                | N= 13, K= 1     |    |    |       | 2 26 10 315.3  |    |    |       |
| 3 72 76 114.4   |    |    |       | 0 157 175 270.0 |    |    |       | 2 182 186 26.4  |    |    |       | 0 76 72 270.0   |    |    |       | 5 72 70 174.3   |    |    |       | N= 12, K= 10    |    |    |                | 0 56 60 90.0    |    |    |       | 3 27 25 287.5  |    |    |       |
| 4 60 49 76.7    |    |    |       | 1 139 146 224.9 |    |    |       | 3 67 63 251.7   |    |    |       | 1 63 64 291.6   |    |    |       | N= 12, K= 3     |    |    |       | 0 131 140 0.0   |    |    |                | 1 35 27 211.0   |    |    |       | N= 13, K= 10   |    |    |       |
| 5 234 14 245.2  |    |    |       | 2 200 202 193.9 |    |    |       | 4 60 67 308.6   |    |    |       | 2 111 118 138.1 |    |    |       | 0 284 6 180.0   |    |    |       | 2 244 13 112.8  |    |    |                | 2 37 30 182.7   |    |    |       | 0 55 50 90.0   |    |    |       |
| N= 10, K= 18    |    |    |       | 4 41 49 16.1    |    |    |       | 5 70 62 256.4   |    |    |       | 3 294 11 325.0  |    |    |       | 2 67 62 221.2   |    |    |       | 4 121 118 137.3 |    |    |                | 1 66 67 310.4   |    |    |       | 2 61 38 95.7   |    |    |       |
| 0 274 25 180.0  |    |    |       | 5 194 194 194.1 |    |    |       | 6 65 61 272.8   |    |    |       | N= 11, K= 16    |    |    |       | 3 124 135 162.3 |    |    |       | 0 102 100 18.4  |    |    |                | N= 13, K= 2     |    |    |       | N= 13, K= 11   |    |    |       |
| 1 30 15 258.9   |    |    |       | 6 187 189 52.7  |    |    |       | 0 57 70 270.0   |    |    |       | 0 264 21 270.0  |    |    |       | 3 124 135 162.3 |    |    |       | N= 12, K= 11    |    |    |                | 0 60 62 270.0   |    |    |       | 0 106 106 90.0 |    |    |       |
| 2 70 71 210.4   |    |    |       | 7 224 18 226.7  |    |    |       | 1 58 56 1.5     |    |    |       | 1 264 10 150.1  |    |    |       | 5 31 23 133.7   |    |    |       | 0 74 72 0.0     |    |    |                | 1 101 97 257.6  |    |    |       | 2 34 24 125.2  |    |    |       |
| 3 85 84 161.8   |    |    |       | N= 11, K= 3     |    |    |       | 2 134 129 309.4 |    |    |       | 2 44 32 132.7   |    |    |       | N= 12, K= 4     |    |    |       | 1 50 50 34.3    |    |    |                | 2 47 50 44.9    |    |    |       | 2 50 39 3.1    |    |    |       |
| 4 284 29 83.4   |    |    |       | 0 90 87 270.0   |    |    |       | 3 124 117 197.5 |    |    |       | 4 111 118 325.4 |    |    |       | 2 39 33 116.4   |    |    |       | 2 39 33 116.4   |    |    |                | 3 39 40 348.0   |    |    |       | N= 13, K= 12   |    |    |       |
| 5 88 85 142.3   |    |    |       | 1 137 130 187.1 |    |    |       | 4 58 61 335.5   |    |    |       | N= 11, K= 17    |    |    |       | 3 254 32 190.4  |    |    |       | 4 234 22 107.9  |    |    |                | 4 62 36 207.5   |    |    |       | 0 85 22 90.0   |    |    |       |
| N= 10, K= 19    |    |    |       | 2 161 168 182.9 |    |    |       | 0 183 200 270.0 |    |    |       | 0 33 40 270.0   |    |    |       | 4 234 22 107.9  |    |    |       | 0 53 51 90.0    |    |    |                | 1 56 62 86.0    |    |    |       | 0 104 104 90.0 |    |    |       |
| 0 264 16 180.0  |    |    |       | 3 17 36 92.9    |    |    |       | 1 128 127 207.8 |    |    |       | 1 42 42 215.5   |    |    |       | 0 28 34 0.0     |    |    |       | 2 77 71 165.1   |    |    |                | 3 294 21 79.5   |    |    |       | 0 205 191 0.0  |    |    |       |
| 1 34 34 144.5   |    |    |       | 4 142 141 324.0 |    |    |       | 2 120 122 46.9  |    |    |       | N= 11, K= 18    |    |    |       | 4 105 102 341.2 |    |    |       | 1 30 19 82.1    |    |    |                | 4 116 117 176.4 |    |    |       | N= 14, K= 0    |    |    |       |
| 2 264 17 40.5   |    |    |       | 5 75 74 223.9   |    |    |       | 3 62 38 238.2   |    |    |       | 3 88 81 246.1   |    |    |       | 5 78 86 202.6   |    |    |       | 3 294 21 79.5   |    |    |                | 0 205 191 0.0   |    |    |       | 1 224 14 270.0 |    |    |       |
| 3 37 36 92.9    |    |    |       | N= 11, K= 4     |    |    |       | 4 125 129 343.9 |    |    |       | N= 11, K= 19    |    |    |       | 0 30 16 180.0   |    |    |       | 4 234 22 107.9  |    |    |                | N= 13, K= 3     |    |    |       | N= 14, K= 1    |    |    |       |
| 4 67 57 46.3    |    |    |       | 0 81 74 90.0    |    |    |       | 5 35 40 384.3   |    |    |       | 0 38 26 90.0    |    |    |       | 1 42 39 234.4   |    |    |       | 0 102 102 270.0 |    |    |                | 1 56 62 86.0    |    |    |       | 0 60 60 0.0    |    |    |       |
| N= 10, K= 20    |    |    |       | 1 150 150 7.5   |    |    |       | 6 81 72 355.7   |    |    |       | 1 77 75 126.0   |    |    |       | 2 274 19 139.9  |    |    |       | 2 77 71 165.1   |    |    |                | 2 34 24 125.2   |    |    |       | 1 30 27 82.9   |    |    |       |
| 0 254 32 180.0  |    |    |       | 2 122 123 281.2 |    |    |       | 0 76 75 270.0   |    |    |       | 2 97 94 230.2   |    |    |       | 4 62 62 91.5    |    |    |       | 1 28 22 184.6   |    |    |                | 3 78 78 72.3    |    |    |       | N= 14, K= 2    |    |    |       |
| 1 254 10 251.2  |    |    |       | 3 120 120 21.4  |    |    |       | 2 73 73 42.7    |    |    |       | 3 67 69 121.0   |    |    |       | 5 40 44 166.2   |    |    |       | 2 34 30 103.7   |    |    |                | 4 24 34 111.4   |    |    |       | 0 92 93 0.0    |    |    |       |
| 2 82 88 134.3   |    |    |       | 4 57 67 204.4   |    |    |       | N= 11, K= 10    |    |    |       | N= 11, K= 10    |    |    |       | 0 24 10 270.0   |    |    |       | 3 84 34 111.4   |    |    |                | N= 12, K= 6     |    |    |       | N= 14, K= 3    |    |    |       |
| 3 80 58 334.3   |    |    |       | 5 45 50 270.7   |    |    |       | N= 11, K= 11    |    |    |       | N= 11, K= 11    |    |    |       | 1 274 33 285.6  |    |    |       | 0 284 21 270.0  |    |    |                | N= 12, K= 13    |    |    |       | N= 14, K= 4    |    |    |       |
| 4 234 6 170.8   |    |    |       | 6 63 65 132.3   |    |    |       | N= 11, K= 12    |    |    |       | N= 11, K= 12    |    |    |       | 2 74 64 350.8   |    |    |       | 1 101 97 257.6  |    |    |                | 0 284 21 270.0  |    |    |       | 1 74 73 175.7  |    |    |       |
| N= 10, K= 21    |    |    |       | 7 57 69 170.8   |    |    |       | N= 11, K= 13    |    |    |       | N= 11, K= 13    |    |    |       | 3 88 81 246.1   |    |    |       | 2 34 24 125.2   |    |    |                | 0 284 21 270.0  |    |    |       | 2 34 24 125.2  |    |    |       |
| 0 24 33 0.0     |    |    |       | 8 63 65 132.3   |    |    |       | N= 11, K= 14    |    |    |       | N= 11, K= 14    |    |    |       | 4 111 118 325.4 |    |    |       | 3 78 78 72.3    |    |    |                | 0 284 21 270.0  |    |    |       | 3 78 78 72.3   |    |    |       |
| 1 244 6 176.1   |    |    |       |                 |    |    |       | N= 11, K= 15    |    |    |       | N= 11, K= 15    |    |    |       | 5 40 44 166.2   |    |    |       | 4 24 34 111.4   |    |    |                | 0 284 21 270.0  |    |    |       | 4 24 34 111.4  |    |    |       |
| 2 244 26 135.9  |    |    |       |                 |    |    |       | N= 11, K= 16    |    |    |       | N= 11, K= 16    |    |    |       | 6 63 65 132.3   |    |    |       |                 |    |    | 0 284 21 270.0 |                 |    |    |       |                |    |    |       |



### 1.3 RESULTS AND DISCUSSION

This crystal structure determination has resolved the ambiguity in the designation of the glycosidic linkage. The correct chemical name for ouabain is 3-O- $\alpha$ -L-rhamnopyranosyl-1 $\beta$ ,3 $\beta$ ,5,11 $\alpha$ ,14,19-hexahydroxy-5 $\beta$ -card 20 (22)-enolide. This agrees with the Klyne rule prediction (31) that cardiac glycoside L-sugars are joined to the aglycone by an  $\alpha$ -glycosidic linkage. The molecular structure is depicted in the stereo-pair of Figure 1.4, drawn with the program ORTEP (39).

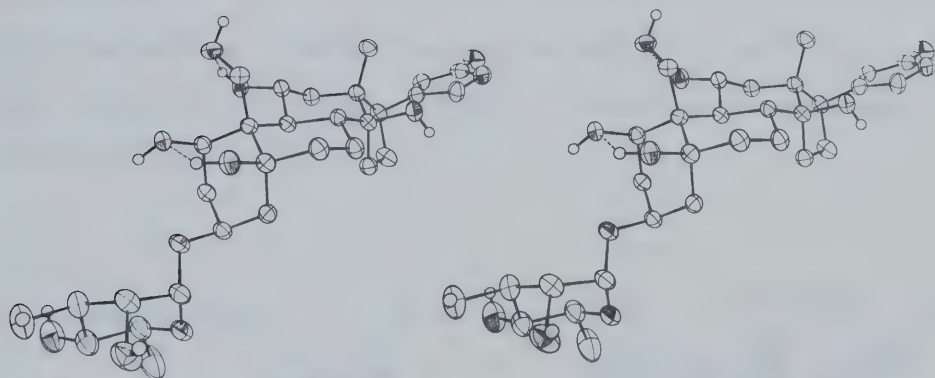


Figure 1.4 The thermal ellipsoids of the anisotropic atoms of the ouabain molecule are scaled to include 35% probability. The oxygen atoms are drawn with one octant of the ellipsoid removed.

The rhamnosyl moiety occurs as a 1-C chair conformation pyranose ring in agreement with the prediction of Reeves (40) and with the crystal structure of  $\alpha$ -L-rhamnose monohydrate (41). This conformation is



sterically more favored than the C-1 conformation which would require both the methyl and 3'-hydroxyl substituents to be axial and on the same side of the six-membered ring.

The overall conformation of the steroid concurs with the conformations observed in the crystal structures of digitoxigenin (18) and strophanthidin (20) which are shown in Figure 1.5. The three axial hydroxyl substituents of the A ring in ouabain form a triangular cluster with this axial orientation stabilized by the presence of the intramolecular hydrogen bond  $O(5)-H(O5)\cdots O(1)$ , indicated by a dashed in Figure 1.4. A second intramolecular hydrogen bond,  $O(11)-H(O11)\cdots O(19)$ , increases the stability of the chair conformation for the B and C rings. The D-ring shows an envelope conformation with the atoms C(13), C(15), C(16) and C(17) lying within 0.006Å of the best least-squares plane for these four atoms.

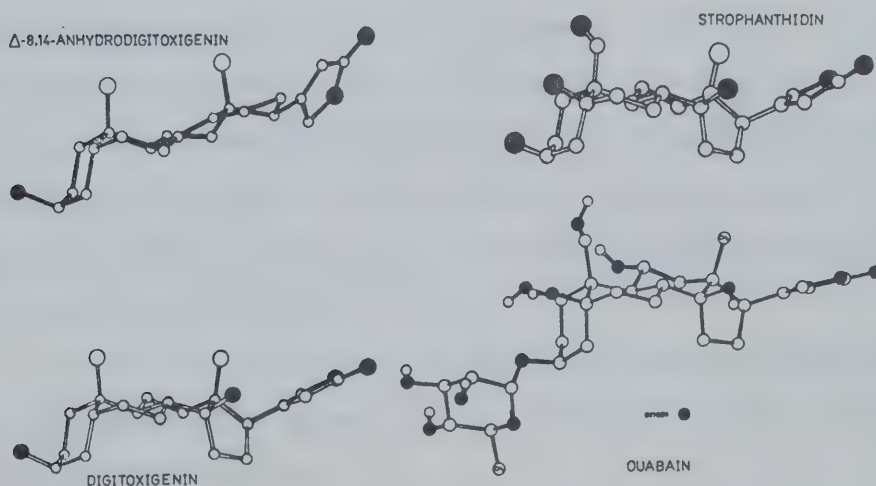


Figure 1.5 A comparison of the molecular conformations observed in the crystal structures of four cardiotonic steroids.



The butenolide ring is also planar. In order to make the orientation of the lactone ring with respect to the D-ring of the steroid agree with that observed in digitoxigenin, a  $164^\circ$  rotation of the butenolide ring about the C(17)-C(20) bond is necessary.

The hydroxyl substituents of the glycoside stabilize the overall conformation of the ouabain molecule by participating in a complex network of hydrogen bonds. Although the locations of the hydrogen atoms of the solvent molecules were not determined, the oxygen-oxygen separations, plus the angles formed between sets of three oxygen atoms, suggest that these atoms are also in this network.

A detailed description of the bonding geometry, conformational analysis, hydrogen bonding and crystal packing are discussed in sections 1.3.1, 1.3.2, 1.3.3 and 1.3.4. Bond distances and valence bond angles are illustrated in Figure 1.6 and 1.7.

It should be emphasized that parameter standard deviations obtained from a block diagonal least-squares refinement tend to be underestimated due to the neglect of inter-atomic correlations. Accordingly, the e.s.d.'s in bond distances and angles, which were obtained from the e.s.d.'s in positional parameters by the independent atom method of Ahmed *et al* (35), may be underestimated by as much as a factor of 2. Average bond angles and bond lengths were calculated using the formula for a biased mean, given in the Preface.

The e.s.d. associated with each weighted mean or simple average was calculated using the equation for the standard deviation of the mean, also listed in the Preface.





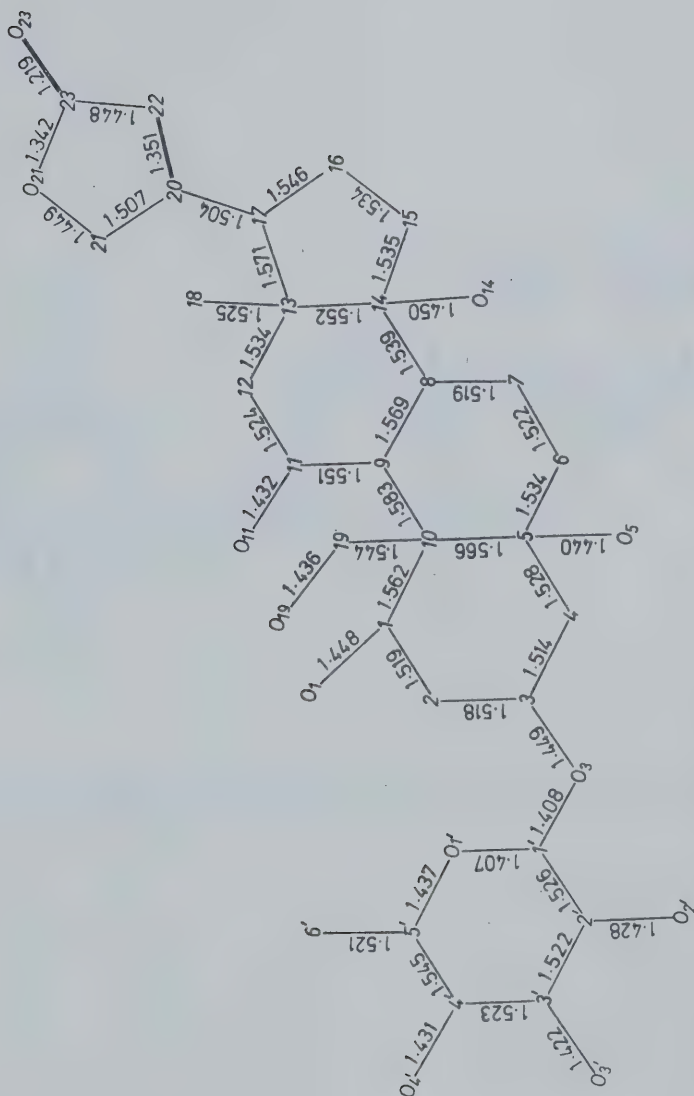


Figure 1.6 Carbon-carbon and carbon-oxygen bond lengths observed in the ouabain molecule. The e.s.d.'s for covalent bond distances range from 0.005 to 0.006 Å for carbon-carbon bonds and 0.004 to 0.006 Å for carbon-oxygen bonds. The e.s.d. of the C(5')-C(6') bond length is 0.008 Å.



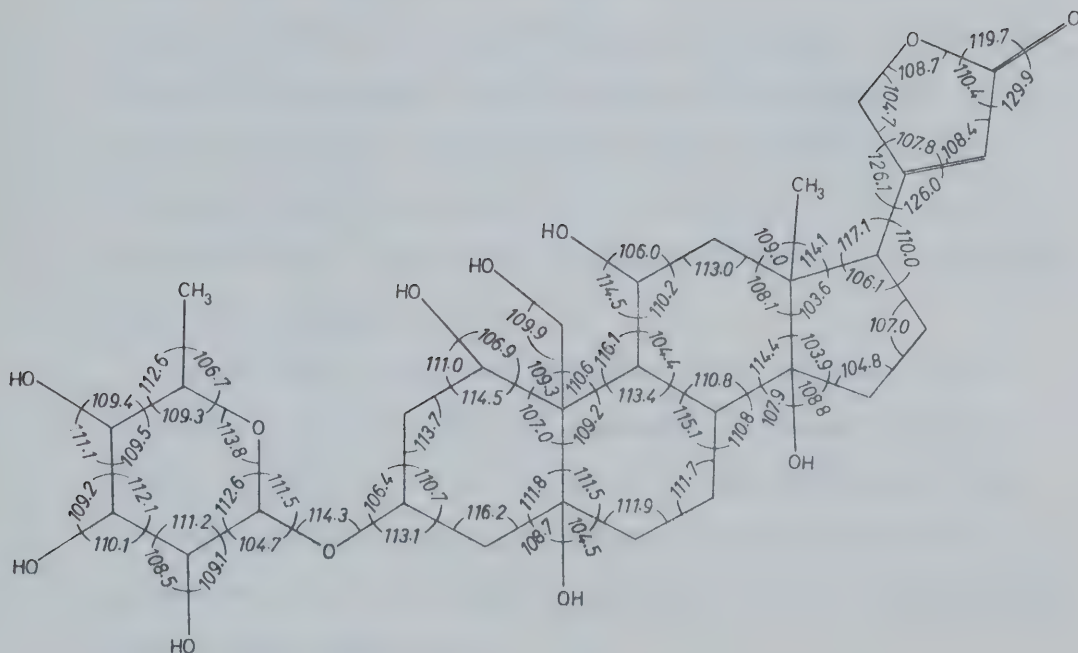


Figure 1.7 Valence bond angles observed in the ouabain molecule. The following angles are missing.

|                      |                    |                       |                    |
|----------------------|--------------------|-----------------------|--------------------|
| C(1) - C(10) - C(9)  | 110.4 <sup>o</sup> | C(12) - C(13) - C(17) | 107.4 <sup>o</sup> |
| C(4) - C(5) - C(6)   | 108.9              | C(14) - C(13) - C(18) | 114.4              |
| O(5) - C(5) - C(10)  | 111.2              | C(8) - C(14) - C(15)  | 115.6              |
| C(5) - C(10) - C(19) | 110.3              | O(14) - C(14) - C(13) | 105.6              |

The e.s.d's for valence bond angles excluding hydrogen atoms range from 0.3 to 0.4<sup>o</sup> in the steroid except in the lactone and rhamnose moieties where the range is 0.4 to 0.5<sup>o</sup>.



### 1.3.1 Bond distances and angles excluding hydrogen atoms

A wide range of carbon ( $sp^3$ )-carbon ( $sp^3$ ) single bonds is found in the aglycone with values ranging from 1.583(5) to 1.514(5)Å. The weighted mean distance for 22 bonds of this type is 1.542(4)Å in agreement with the carbon-carbon single bond distance of 1.5445(1)Å observed in diamond (6). This variation, while significant, is not unusual in structures of this kind. Similar phenomena have been observed in the crystal structures of digitoxigenin (DTG) (18), gutierolide (42),  $\Delta$ -8,14-anhydrodigitoxigenin (ADTG) (19) and methyl melaleucate iodoacetate (43).

Each of these structures contains a highly substituted fused ring system. In the first three compounds, the stereochemistry of the A/B ring fusion is *cis*. The length of the  $C(sp^3)$ - $C(sp^3)$  bond increases with the number of non-hydrogen substituents to each atom of the bond. Thus the longest bonds are often, but not always, those common to two or more rings.

Gilardi and Karle (19) suggest that this variation is the result of three steric factors which are:-

- (i) Gauche interactions between non-bonded neighboring substituents
- (ii) Van der Waals interactions
- (iii) The effect of ring closure

To illustrate the influence of (i) on the distances observed in the aglycone, the Newman projections of those carbon-carbon bonds which contain two or three non-hydrogen substituents to each atoms of the bond are depicted in Figure 1.8. Torsion angles about ring bonds not included in Figure 1.8 are listed in Table 1.8 using the sign convention of Klyne and Prelog (44).



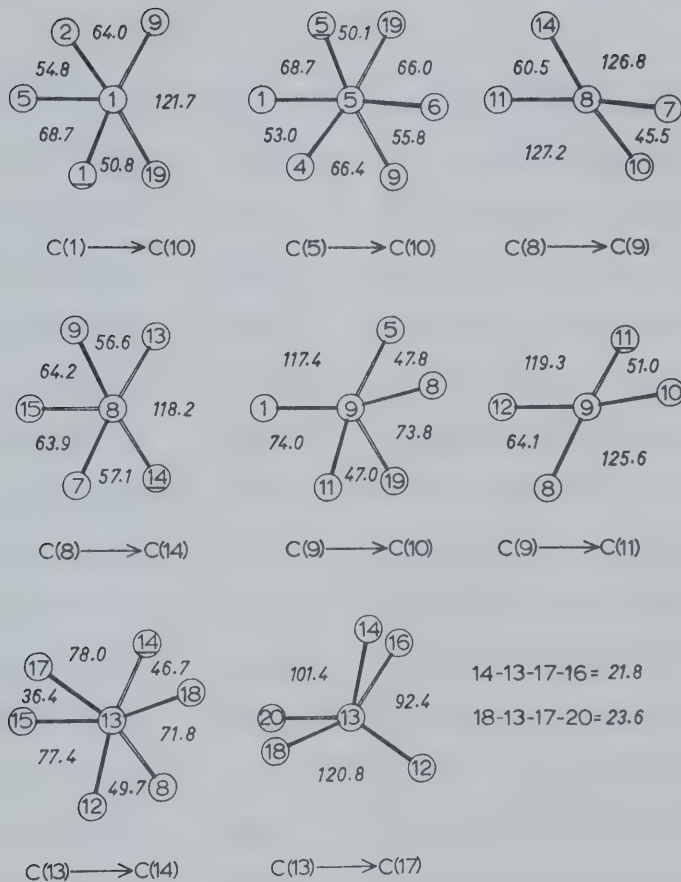


Figure 1.8 Projections down steroid ring bonds containing two or more non-hydrogen atoms to each of the bonded atoms. Carbon atoms are designated by their atom numbers with the numbers of oxygen atoms underlined. Blacked-out bonds are to the uppermost atom in the projection.





Table 1.8

Torsion angles for ring bonds not shown in Figure 1.8

| A-B-C-D *             | $\tau$ | A-B-C-D                 | $\tau$ |
|-----------------------|--------|-------------------------|--------|
| C(10)-C(1)-C(2)-C(3)  | +54.2  | C(1) -C(10)-C(19)-O(19) | -44.0  |
| O(1) -C(1)-C(2)-C(3)  | -67.0  | C(5) -C(10)-C(19)-O(19) | -161.4 |
| C(1) -C(2)-C(3)-C(4)  | +48.1  | C(9) -C(10)-C(19)-O(19) | +77.7  |
| C(1) -C(2)-C(3)-O(3)  | +75.2  | C(9) -C(11)-C(12)-C(13) | -63.7  |
| C(2) -C(3)-C(4)-C(5)  | +49.2  | O(11)-C(11)-C(12)-C(13) | +171.9 |
| O(3) -C(3)-C(4)-C(5)  | -70.1  | C(11)-C(12)-C(13)-C(14) | +52.7  |
| C(3) -C(4)-C(5)-C(6)  | -177.4 | C(11)-C(12)-C(13)-C(17) | +163.9 |
| C(3) -C(4)-C(5)-C(10) | -53.8  | C(11)-C(12)-C(13)-C(18) | -72.1  |
| C(3) -C(4)-C(5)-O(5)  | +69.3  | C(13)-C(14)-C(15)-C(16) | +37.4  |
| C(4) -C(5)-C(6)-C(7)  | +62.8  | O(14)-C(14)-C(15)-C(16) | -74.7  |
| O(5) -C(5)-C(6)-C(7)  | +178.7 | C(8) -C(14)-C(15)-C(16) | +163.7 |
| C(10)-C(5)-C(6)-C(7)  | -61.1  | C(14)-C(15)-C(16)-C(17) | -23.6  |
| C(5) -C(6)-C(7)-C(8)  | +55.7  | C(15)-C(16)-C(17)-C(13) | +0.9   |
| C(6) -C(7)-C(8)-C(9)  | -48.1  | C(15)-C(16)-C(17)-C(20) | +128.5 |
| C(6) -C(7)-C(8)-C(14) | -174.9 | C(13)-C(17)-C(20)-C(21) | +60.2  |

\* A positive torsion angle ( $\tau$ ) indicates that a clockwise rotation of the uppermost (A-B) bond about the B-C axis will make A-B coincide with C-D (44).



Figure 1.8 shows that the substituents to the C(13)-C(17) bond (1.571 Å) are arranged in an eclipsed conformation. This results in a large repulsive interaction between the two non-bonded atom pairs C(14), C(16) and C(18), C(20). The large C(9)-C(10) bond length is hardly surprising in that each of the five methylene substituents attached to this atom pair is bonded to at least one additional methylene substituent. The substituents to the C(13)-C(14) bond exhibit a semi-eclipsed conformation. However the magnitude of the interaction between O(14) and C(18) will be less important due to the smaller Van der Waals radius of oxygen (1.4 Å) compared to methylene (2.0 Å).

In contrast, the shortest C(sp<sup>3</sup>)-C(sp<sup>3</sup>) bonds in the steroid nucleus are those in which the two carbon atoms are bonded to at least three hydrogen atoms. Since hydrogen has a smaller Van der Waals radius than a methylene substituent, gauche interactions of the type carbon...hydrogen or hydrogen...hydrogen will only be appreciable when the torsion angle between the interacting pair of atoms approaches 0° as is observed for the C(14)-C(15), C(15)-C(16) and C(16)-C(17) bonds in the D ring. Accordingly, the shortest C(sp<sup>3</sup>)-C(sp<sup>3</sup>) single bonds in the steroid skeleton are C(1)-C(2), 1.519 Å; C(2)-C(3), 1.518 Å; C(3)-C(4), 1.514 Å, C(6)-C(7), 1.522 Å and C(7)-C(8), 1.519 Å. Each of these bonds is significantly shorter than those bonds whose Newman projections are shown in Figure 1.8.

Van der Waals repulsions between hydrogen atoms will occur when their separations are less than 2.34 Å, the sum of the Van der Waals radii for hydrogen, given by Kitaigorodski (46).



The large values of the C(9)-C(10) and C(8)-C(9) bonds may be necessary in order to achieve the separation of 2.12 Å between H(91) and H(22); 2.28 Å between H(91) and H(42) and 2.05 Å between H(91) and H(152). Two additional short hydrogen-hydrogen contacts which occur as a result of the cis-ring fusions are H(42)···H(72) (1.99 Å) and H(72)···H(151) (2.34 Å). Although the methylene and methine hydrogen atom positions were calculated, and not determined by difference Fourier techniques, the H(91) substituent will always be somewhat crowded in the digitalis structures due to the stereochemistry of the three ring junctions.

The effect of ring closure is to limit the conformational flexibility of the structure in a way so that repulsive Van der Waals forces between pairs of non-bonded atoms will result in a modification of bond lengths and bond angles.

Several crystal structures containing an  $\alpha$ ,  $\beta$ -unsaturated  $\gamma$ -lactone have been determined during the last seven years. These structures include ascorbic acid (47), methyl-2-acetonyl-3-ethyl-4-methoxy-5-oxo-dihydro-2H-furan-2-carboxylate (48), ADTG (19), DTG (18), gutierolide (42) and ouabain tetrahydrate. The bond angles and distances for the butenolide rings of the last four structures are listed in Table 1.9 according to the designation of Figure 1.9.

The two C(sp<sup>3</sup>)-C(sp<sup>2</sup>) single bond distances, A and F, agree with the average C(sp<sup>3</sup>)-C(sp<sup>2</sup>) distance of 1.505(5) Å listed by Sutton (6). Correction of the ouabain C(23)-O(23) distance of 1.219 Å (G in Figure 1.8) for 'riding motion' (49) gave a value of 1.254 Å. Mo and Sivertson (48) state that a carbonyl bond distance of 1.215 Å (uncorrected for thermal motion) is typical of lactones



in which the carbonyl oxygen participates as a hydrogen bond acceptor and that this value is shortened by about  $0.015 \text{ \AA}$  in structures in which the oxygen atom is not a hydrogen bond acceptor. In both gutierolide (42) and ADTG (19), the carbonyl oxygen atom does not participate in hydrogen bonding. In DTG (18), Karle and Karle state that the carbonyl oxygen atom participates in an intermolecular hydrogen bond with the 3-hydroxyl of a symmetry related molecule. However, the observed oxygen...oxygen separation was  $2.94 \text{ \AA}$  and neither of the hydroxyl hydrogen atoms were found in that structure.

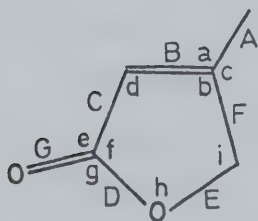


Figure 1.9 The designation of the bond distances and bond angles listed in Table 1.9.

The ester linkage of the butenolide moiety in each structure listed in Table 1.9, shows a characteristic disproportionation of the two carbon-oxygen single bonds (D and E in Figure 1.9). Merlino (50) has found that the disproportionation of the C-O bond lengths is a characteristic of the ester linkage in general. He lists a range of  $1.365(6)$  to  $1.323(4) \text{ \AA}$  for the shorter bond (D) and  $1.360(5)$  to  $1.482(3) \text{ \AA}$  for the longer bond (E) for 13 crystal structures containing the ester moiety. The C(23)-O(21) and O(21)-C(21) distances





Table 1.9

Bond lengths and bond angles observed in four  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone moieties. The designation of bond distances and valence bond angles is shown in Figure 1.9.

|                            | Gutierolide        | Ouabain                  | ADTG        | DTG         |
|----------------------------|--------------------|--------------------------|-------------|-------------|
| A                          | 1.507 $\text{\AA}$ | 1.504                    | 1.495       | 1.503       |
| B                          | 1.316              | 1.351                    | 1.317       | 1.358       |
| C                          | 1.460              | 1.448                    | 1.457       | 1.463       |
| D                          | 1.336              | 1.342                    | 1.365       | 1.391       |
| E                          | 1.452              | 1.449                    | 1.438       | 1.451       |
| F                          | 1.487              | 1.507                    | 1.499       | 1.484       |
| G                          | 1.212              | 1.219                    | 1.202       | 1.178       |
| a                          | 130.5 $^\circ$     | 126.0                    | 131.1       | 130.6       |
| b                          | 108.6              | 107.8                    | 108.0       | 109.4       |
| c                          | 120.9              | 126.1                    | 120.9       | 120.0       |
| d                          | 109.4              | 108.4                    | 108.4       | 108.2       |
| e                          | 129.7              | 129.9                    | 131.3       | 133.0       |
| f                          | 108.5              | 110.4                    | 108.5       | 108.5       |
| g                          | 121.7              | 119.7                    | 120.2       | 118.5       |
| h                          | 109.2              | 108.7                    | 108.5       | 108.7       |
| i                          | 104.3              | 104.7                    | 105.3       | 105.1       |
| e.s.d. of bond distances   |                    | 0.003-0.004 $\text{\AA}$ | 0.005-0.006 | 0.006 0.012 |
| e.s.d. of interbond angles |                    | 0.2-0.3 $^\circ$         | 0.3-0.4     | 0.7 0.9     |



of 1.342 and 1.449(4) Å in ouabain fall within each of these ranges.

Each of the butenolide moieties is a five-membered planar ring which requires the internal bond angles of the ring to have an average value of 108°. In order to arrive at this value the  $sp^2$  disposition of the bonding orbitals for four of the five ring atoms must be reduced from the theoretical value of 120° by 10 to 12° to arrive at their present values. In each structure the angle  $\angle$  between the ring bonds to the  $sp^3$  carbon atom has been reduced from a theoretical value of 109.5° to values between 104.3 and 105.3°. The O=C-C angle is between 8.0 and 14.5° larger than the O=C-O valence angle. This difference is also observed in saturated  $\gamma$ -lactones and is somewhat smaller for non-cyclic molecules containing ester linkages.

The six carbon-oxygen single bonds to the ouabain sterol nucleus have a weighted mean value of 1.443(3) Å and range from 1.450 Å to 1.432 Å compared to the literature value of 1.426(5) Å (6). Long carbon-oxygen distances of 1.458 and 1.462(12) Å are observed for the C(3)-O(3) and C(14)-O(14) bond in DTG (18) and 1.453(5) Å for the C(3)-O(3) bond in ADTG (19). The bond lengthening may be a result of steric interactions of the oxygen atoms with atoms of the steroid nucleus.

The bond distances observed in the rhamnosyl moiety of ouabain are almost identical to those observed in  $\alpha$ -L-rhamnose monohydrate (41). The mean C( $sp^3$ )-C( $sp^3$ ) bond length is 1.527(4) Å and the mean C-O bond length is 1.422(5) Å. A comparison of the bond distances and interbond angles for the two structures (excluding hydrogen atoms) is given in Table 1.10.



Table 1.10

Bond distances and bond angles observed in  $\alpha$ -L-rhamnose monohydrate (41) and the rhamnosyl moiety of ouabain.

| Covalent bond distances |   |                    | Interbond angles (degrees) |       |       |       |                         |                          |
|-------------------------|---|--------------------|----------------------------|-------|-------|-------|-------------------------|--------------------------|
| A                       | B | A-B (Å)<br>ouabain | A-B<br>rhamnose            | i     | j     | k     | $\angle ijk$<br>ouabain | $\angle ijk$<br>rhamnose |
| C(1')-C(2')             |   | 1.526(6)           | 1.526(3)                   | C(1') | C(2') | C(3') | 111.2(4)                | 110.5(3)                 |
| C(2')-C(3')             |   | 1.522(6)           | 1.522(3)                   | C(2') | C(3') | C(4') | 112.1                   | 108.9                    |
| C(3')-C(4')             |   | 1.523(6)           | 1.521(3)                   | C(3') | C(4') | C(5') | 109.5                   | 109.1                    |
| C(4')-C(5')             |   | 1.545(7)           | 1.545(3)                   | C(4') | C(5') | O(1') | 109.3                   | 108.5                    |
| C(5')-C(6')             |   | 1.521(8)           | 1.509(4)                   | C(5') | O(1') | C(1') | 113.8                   | 115.0                    |
|                         |   |                    |                            | O(1') | C(1') | C(2') | 112.6                   | 110.0                    |
| C(1')-O(1')             |   | 1.407(5)           | 1.422(3)                   | C(2') | C(1') | O(3)  | 104.7                   | 108.6                    |
| C(2')-O(2')             |   | 1.428(6)           | 1.429(3)                   | O(1') | C(1') | O(3)  | 111.5                   | 111.9                    |
| C(3')-O(3')             |   | 1.422(6)           | 1.419(3)                   | O(2') | C(2') | C(1') | 109.1                   | 107.7                    |
| C(4')-O(4')             |   | 1.431(6)           | 1.424(3)                   | O(2') | C(2') | C(3') | 108.5                   | 110.9                    |
| C(5')-O(1')             |   | 1.437(6)           | 1.444(3)                   | O(3') | C(3') | C(2') | 110.1                   | 106.7                    |
| C(1')-O(3)              |   | 1.408(5)           | 1.401(3)                   | O(3') | C(3') | C(4') | 109.2                   | 113.6                    |
|                         |   |                    |                            | O(4') | C(4') | C(3') | 111.1                   | 108.9                    |
|                         |   |                    |                            | O(4') | C(4') | C(5') | 109.4                   | 110.7                    |
|                         |   |                    |                            | C(4') | C(5') | C(6') | 112.6                   | 113.4                    |
|                         |   |                    |                            | O(1') | C(5') | C(6') | 106.7                   | 106.8                    |



The anomeric C(1')-oxygen distances (1.408, 1.407(5) Å) are significantly shorter than the C(5')-O(1') distance of 1.437(5) Å. A similar disproportionation of carbon-oxygen bond distances is observed in methyl- $\alpha$ -D-glucoside (51) and methyl  $\beta$ -maltoside (52). Each structure contains an axial glycosidic bond. In two pyranoside structures (53) containing an equatorial glycosidic linkage, the glycosidic carbon-oxygen bond length is significantly shorter than the two carbon-ring oxygen bond lengths which are similar.

### 1.3.2 Ring Conformations

Although to a first approximation the four 6-membered rings of the ouabain molecule exhibit chair conformations, the occurrence of internal ring valence angles greater than  $109.5^\circ$  and torsion angles about ring bonds differing from  $\pm 60^\circ$  indicates each ring to be somewhat distorted from ideality. If the chair conformation is exact, alternate bonded atom pairs should be planar with the third and sixth atoms of the ring equally exoplanar but on opposite sides of the 4-atom plane. Also, torsion angles contained by four adjacent atoms should alternate about the ring with values of  $+60^\circ$  and  $-60^\circ$ .

All sets of four atom planes of the above kind were calculated for each 6-membered ring. These are listed in Table 1.11 in the form  $ax + by + cz = P$ .

Associated with each plane is the value Chi-squared ( $\chi^2$ ) which is defined by the equation

$$\chi^2 = \sum_i (\Delta/\sigma)^2 \quad \text{where}$$

$\Delta$  is the deviation of atom  $i$  from the plane and





$\sigma$  is the e.s.d. for each deviation, derived from a knowledge of the e.s.d.'s of the atomic coordinates. Low values of  $\chi^2$  indicate a planar system (54).

It can be seen that the distortion of the A ring from an ideal chair conformation is principally associated with the inequality of the perpendicular distances of C(3) and C(10) from the four atom plane defined by C(1), C(2), C(4) and C(5). The atom O(3) may have been forced more into the four atom plane in order to achieve the separation O(3)...O(1), 2.848 Å and O(3)...O(5), 2.961 Å. A second source of ring distortion is the inequality of the bond distances in the A ring. In order to achieve an undistorted chair conformation all distances between bonded atoms in the ring should be equal.

The B ring of the steroid is considerably distorted from an ideal chair conformation probably as the result of steric hindrance between H(91) and the 2 $\alpha$ , 4 $\alpha$ , 12 $\alpha$  and 15 $\alpha$ -hydrogen atoms of the adjacent rings. The set of four atoms most closely approximating a plane is C(6), C(7), C(9), C(10). The small values of the torsion angle C(6)-C(7)-C(8)-C(9) (-48.1°) and C(7)-C(8)-C(9)-C(10) (45.5°) substantiate the observed ring flattening at the B/C ring junction. The C ring also contains a twisting distortion with the C(9)-C(11) and C(13)-C(14) bonds somewhat askew with respect to each other.

The D ring exhibits an  $\alpha$ -envelope conformation (55) with C(13), C(15), C(16) and C(17) all within 0.006 Å of the best least-squares plane for these four atoms. C(14) is 0.577 Å out of this plane and on the same side of the plane as C(18). A similar  $\alpha$ -envelope



conformation is observed in DTG (18) where the exoplanar deviation of C(14) is 0.53 Å. The D-ring conformation in ADTG (19) is also an envelope. The atoms C(13) to C(16) are coplanar with C(17) 0.635 Å out of the plane on the side opposite C(18). A change in the D-ring conformation in going from DTG to ADTG is expected because the hybridization of the C(14) bonding orbitals changes from  $sp^3$  to  $sp^2$ .

In ouabain, the lactone ring atoms and O(23) are coplanar. The equation of the plane is  $-0.4290x + 0.8830y - 0.1903z = 11.9839$ . The exoplanar deviations of the ring members are listed in Table 1.11. The exoplanar deviation of C(17) is 0.103 Å. In DTG the same six atoms are within 0.009 Å of being coplanar. The exoplanar deviation of C(17) is 0.014 Å. In ADTG, all seven atoms are coplanar and the maximum exoplanar deviation is 0.02 Å.

The rhamnopyranose ring exhibits a chair conformation which is somewhat flattened in the vicinity of C(2) and C(3). This flattening is readily seen in Figure 1.10 which shows the Newman projections for ring bonds in the rhamnose moiety. Ring puckering is maximal at C(5'). Table 1.11 shows that the atoms C(1'), C(3'), C(4') and O(1') are coplanar with a maximum exoplanar deviation of 0.013 Å.

The Newman projections down the C(3)-O(3) and O(3)-C(1') bonds indicate the atoms C(2), C(3), O(3), C(1') and C(2') form an extended chain with alternating bonded atom pairs approximately trans with respect to each other. This arrangement allows for a maximal separation between the hydrogen atoms of the pyranose ring and the  $2\beta$ ,  $4\beta$  and  $3\alpha$ -hydrogens of the sterol nucleus.



Table 1.11

Four atom least-squares planes. Each equation has the form  $ax + by + cz = P$  where a, b and c are the direction cosines of the plane with respect to the x, y and z axes of the unit cell.

| Planar Coefficients X 10 <sup>4</sup> |      |       |       | Exoplanar Distances (Å X 10 <sup>3</sup> ) |         |       |       |       |       |       |                |
|---------------------------------------|------|-------|-------|--|---------|-------|-------|-------|-------|-------|----------------|
|                                       | a    | b     | c     | P  | C(1)    | C(2)  | C(3)  | C(4)  | C(5)  | C(10) | χ <sup>2</sup> |
| Ring A                                |      |       |       |  |         |       |       |       |       |       |                |
|                                       | i    | -3502 | -5152 | -7823                                      | 126904  | 1     | 586*  | 1     | -1    | -691* | 0.2            |
|                                       | ii   | -2223 | -8617 | -4561                                      | -168222 | 641*  | 26    | -590* | 21    | -20   | 153.9          |
| iii                                   | 1189 | -6516 | -7492 | -117905                                    | 23      | -599* | -26   | -24   | 651*  | 21    | 153.3          |
| Ring B                                |      |       |       |  |         |       |       |       |       |       |                |
|                                       | iv   | -4326 | 8839  | -1776                                      | 107583  | 49    | 610*  | 50    | -46   | -657* | 754.9          |
|                                       | v    | -7484 | 5607  | -3542                                      | 19511   | 691*  | -23   | -552* | -18   | 18    | 118.6          |
| vi                                    | 7412 | -6585 | -1300 | -51515                                     | -28     | 683*  | 35    | -29   | -587* | 26    | 252.5          |
| Ring C                                |      |       |       |  |         |       |       |       |       |       |                |
|                                       | vii  | -5194 | 8287  | -2085                                      | 89160   | -28   | 28    | -757* | -38   | 33    | 268.5          |
|                                       | viii | -8388 | 4032  | -3658                                      | -23025  | 19    | 790*  | -21   | 24    | -643* | 116.8          |
| ix                                    | 8228 | -5426 | -1691 | -32394                                     | 716*    | -48   | 58    | -676* | -59   | 55    | 845.5          |
| Rhamnose                              |      |       |       |  |         |       |       |       |       |       |                |
|                                       | x    | -3973 | 3228  | -8591                                      | 43787   | -615* | -37   | 35    | 667*  | 0(1') | 266.2          |
|                                       | xi   | 1060  | 2288  | -9677                                      | 50253   | -11   | 604*  | 10    | -13   | -693* | 19.8           |
| xii                                   | -372 | 6656  | -7454 | 115781                                     | 24      | -23   | -607* | 30    | -32   | 625*  | 129.3          |
| Ring D                                |      |       |       |  |         |       |       |       |       |       |                |
|                                       | xiii | 9283  | 2867  | -2367                                      | 125141  | 2     | 577*  | -3    | 6     | -5    | 3.7            |

\* atom not included in least-squares plane calculation



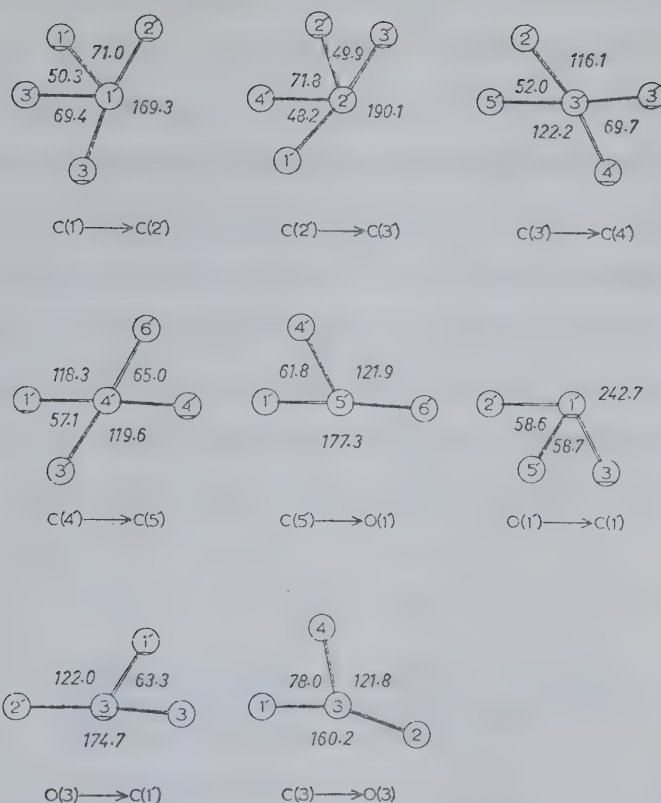


Figure 1.10 Newman projections for ring bonds of the rhamno-pyranosyl moiety using the convention given for Figure 1.8.

James (56) has observed that in axial pyranose glycosides such as methyl  $\beta$ -maltoside (52), methyl  $\alpha$ -D-glucoside (51) and cell-obiose (51), the glycosidic O-R bond is trans with respect to the C(1')-C(2') bond. This phenomenon is also observed in the ouabain structure where the C(3)-O(3)-C(1')-C(2') torsion angle is  $174.7^\circ$ .





### 1.3.3 Hydrogen Bonding

The hydrogen bonds observed in the structure of ouabain tetrahydrate fall into two categories: carbon-hydrogen covalent bonds and oxygen-hydrogen bonds. The carbon-hydrogen bond lengths for methylene and methine hydrogen atoms were arbitrarily set to a value of  $1.04 \text{ \AA}$  with the exception of the C(2)-H(22) bond distance which was accidentally set to  $0.99 \text{ \AA}$ . The carbon-hydrogen bond distances for the two methyl substituents are listed in Table 1.12 and have a simple average value of  $0.89 \text{ \AA}$ . Both sets of methyl hydrogen atoms are ordered and arranged in a staggered conformation with respect to the carbon-carbon bonds to the adjacent carbon atom.

Table 1.12

Carbon-hydrogen bond lengths for hydrogen atoms whose positions were determined by difference Fourier techniques.

|              | Distance ( $\text{\AA}$ ) |              | Distance ( $\text{\AA}$ ) |
|--------------|---------------------------|--------------|---------------------------|
| C(18)-H(181) | 1.03                      | C(6')-H(61') | 0.75                      |
| C(18)-H(182) | 0.79                      | C(6')-H(62') | 1.01                      |
| C(18)-H(183) | 0.91                      | C(6')-H(63') | 0.83                      |

Details of oxygen-hydrogen bonds are listed in Table 1.13. The average oxygen-hydrogen covalent bond length is  $0.86 \text{ \AA}$  which agrees with the average of  $0.87 \text{ \AA}$  observed in  $\alpha$ -L-rhamnose monohydrate (41), and is typical of values observed in X-ray diffraction studies (57). Each hydroxyl hydrogen atom participates in a hydrogen bond to an alcoholic or water oxygen atom. The oxygen...oxygen separations are very similar and range from  $2.682$  to  $2.771 \text{ \AA}$  with a weighted mean



of 2.735 (10) Å. This agrees with the average O...O separation of 2.74 Å tabulated by Donohue (58) for 27 O-H...O hydrogen bonds.

The C-O-H angles are approximately tetrahedral and range from 114 to 100°. The O-H...O angles range from near linear values to the appreciably bent angles of 135 and 142° in the two intramolecular hydrogen bonds. Although Hamilton (59) reports a distribution of O-H...O angles ranging from 180° to 135° with a mean value of 165° from neutron diffraction studies, both intramolecular hydrogen bond O-H...O angles are near the lower limit of this range. However, this is not unexpected as the geometry of the steroid fixes the locations of the oxygen atoms O(1), O(5) and O(11).

Although intramolecular hydrogen bonds have been reported for the half-salts of 1,4-dibasic carboxylic acids (60) or between the two sugar moieties of a disaccharide (cellobiose (52)), relatively few intramolecular hydrogen bonds have been reported between 1,3-diaxial substituents of cycloalkane containing structures. One example of this kind of hydrogen bonding is observed in gutierolide (42) where a hydroxyl is hydrogen bonded to an ether oxygen. Both are axial substituents attached to a five membered cyclic acetal. The O...O separation is 2.715(3) Å and the O-H...O angle is 132°.

Gatehouse and Poppleton (61) report an intramolecular hydrogen bond in methyl- $\alpha$ -D-altopyranoside where the equatorial O(4) oxygen is hydrogen bonded to the axial 3-hydroxyl substituent. The O...O separation is 2.713 Å and the O-H...O angle is 119°. The O-H covalent bond length is 0.83 Å.

Although the hydrogen atoms of the four solvent molecules were not located, several O...O contacts less than 3.00 Å were observed



Table 1.13

## Oxygen-Hydrogen Bonding Distances and Angles

| h     | i                   | j      | k                 | $\ell$    | D ij                               | D jk                               | D ik                                   | $\angle hij$        | $\angle ijk$        | $\angle jk\ell$ |
|-------|---------------------|--------|-------------------|-----------|------------------------------------|------------------------------------|--|---------------------|---------------------|-----------------|
| C(1)  | O(1)                | H(O1)  | O(3')ii           | C(3')ii   | 0.89 $\overset{\circ}{\text{\AA}}$ | 1.93 $\overset{\circ}{\text{\AA}}$ | 2.771(4) $\overset{\circ}{\text{\AA}}$ | 108 $^\circ$        | 156 $^\circ$        | 125 $^\circ$    |
| C(5)  | O(5)                | H(O5)  | O(1) i            | C(1) i    | 0.88                               | 1.99                               | 2.682(4)                               | 114                 | 135                 | 100             |
| C(11) | O(11)               | H(O11) | O(19)i            | C(19)i    | 0.90                               | 1.97                               | 2.738(4)                               | 108                 | 142                 | 115             |
| C(14) | O(14)               | H(O14) | O(W2)i            | -         | 0.79                               | 1.97                               | 2.753(7)                               | 113                 | 171                 | -               |
| C(19) | O(19)               | H(O19) | O(5) iiii         | C(5) iiii | 0.91                               | 1.83                               | 2.736(4)                               | 100                 | 171                 | 126             |
| C(2') | O(2')               | H(O2') | O(11)iv           | C(11)ii   | 0.96                               | 1.81                               | 2.732(5)                               | 108                 | 160                 | 128             |
| C(3') | O(3')               | H(O3') | O(S1)v            | -         | 0.91                               | 2.02                               | 2.753(11)                              | 112                 | 137                 | -               |
| C(4') | O(4')               | H(O4') | O(S1)vi           | -         | 0.66                               | 2.24                               | 2.712(12)                              | 106                 | 130                 | -               |
| i     | x,                  | y,     | z                 |           |                                    |                                    | iv                                     | x,                  | y,                  | -1 + z          |
| ii    | $\frac{1}{2} - x,$  | 1 - y, | $\frac{1}{2} + z$ |           |                                    |                                    | v                                      | 1 - x,              | $\frac{1}{2} - y,$  | -1 + z          |
| iii   | $1\frac{1}{2} - x,$ | 1 - y, | $\frac{1}{2} + z$ |           |                                    |                                    | vi                                     | $-\frac{1}{2} - x,$ | $1\frac{1}{2} - y,$ | 1 + z           |



which infers the oxygen atoms O(W1), O(W2) and O(S2) to be linked together by hydrogen bonds. These short interatomic contacts are listed in Table 1.14.

Table 1.14

Close O...O interatomic contacts  
not listed in Table 1.13.

| Atom A | Atom B  | Separation Å |
|--------|---|--------------|
| O(W1)i | O(W2)i  | 2.82(2)      |
| O(W1)i | O(S2)i  | 2.44(3)      |
| O(W1)i | O(2')ii   | 2.94(2)      |
| O(W2)i | O(23)iii  | 2.80(1)      |
| O(S2)i | O(14)iv   | 2.96(2)      |
| i      | x, y, z   |              |
| ii     | $\frac{1}{2} + x$ , $1\frac{1}{2} - y$ , -z     |              |
| iii    | x, y, -1 + z                                    |              |
| iv     | $-\frac{1}{2} + x$ , $1\frac{1}{2} - y$ , 1 - z |              |

#### 1.3.4 The Crystal packing

The arrangement of the ouabain and solvent molecules in the unit cell is shown in Figure 1.11 and Figure 1.12.





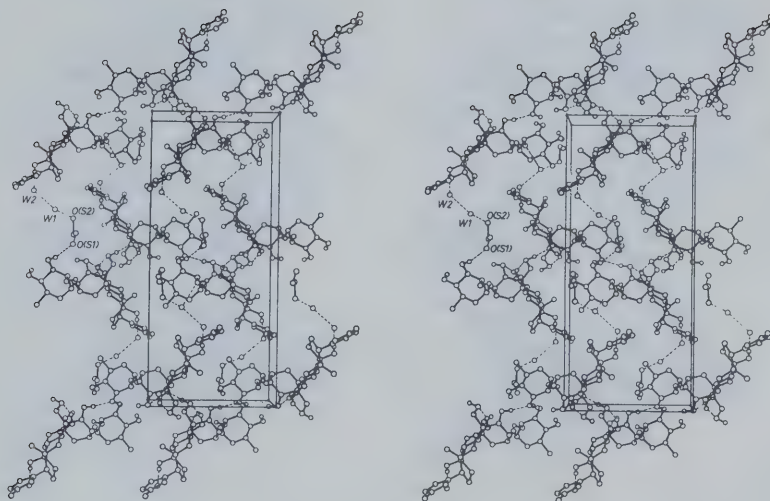


Figure 1.11 The contents of the unit cell for ouabain tetrahydrate viewed with the z-axis of the crystal pointing into the page. The x-axis extends to the right and the y-axis points towards the bottom of the page. The origin of the unit cell corresponds to the upper left hand corner of the enclosure.

The crystal consists of infinite chains of ouabain molecules held together by (i) intermolecular hydrogen bonds between ouabain molecules and (ii) hydrogen bonds between the several ouabain molecules and oxygen atoms of the solvent molecules of crystallization. The solvent molecules are joined together by hydrogen bonds (listed in Table 1.14) and are contained within a sausage-shaped channel approximately  $6 \text{ \AA}$  in diameter.



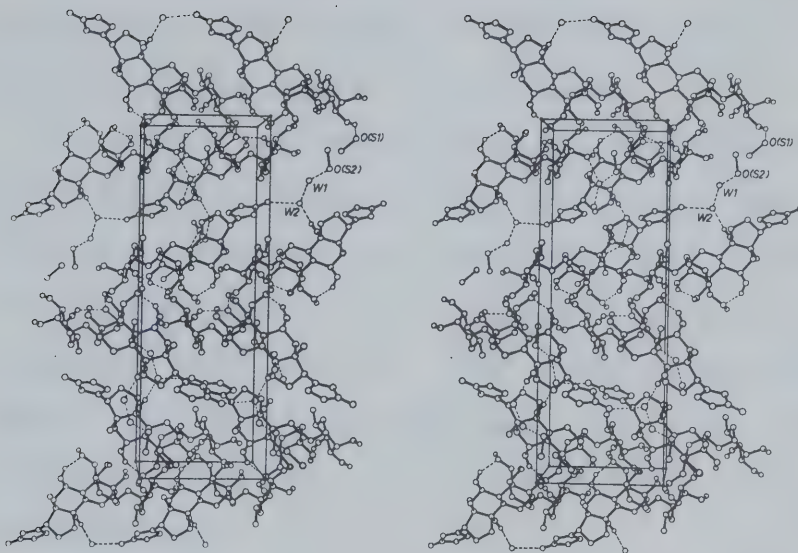


Figure 1.12 The contents of the unit cell for ouabain tetrahydrate viewed along the x-axis. The z-axis extends to the right with the y-axis pointing towards the top of the page. The origin of the unit cell corresponds to the lower left hand corner of the enclosure.

Infinite chains resulting from type (i) interactions are as follows. A hydrogen bond exists between the O(2') hydroxyl and O(11) of all ouabain molecules related by a unit cell translation in the z-direction. This is readily seen in Figure 1.12. Two other infinite chains of ouabain molecules are parallel to the screw diad axis at  $1/4, 0, z$  and  $3/4, 0, z$ . The hydrogen bonds which link the ouabain molecule together are  $H(01) \cdots O(3')$  and  $H(019) \cdots O(5)$  where



O(3') and H(019) are in the same molecule and H(01) and O(5) are in two molecules related to each other by a unit cell translation in the x-direction. (These chains are indicated by dashed lines in Figure 1.11). Thus, the infinite chains parallel to z are cross-linked by hydrogen bonds in the x-direction.

The terminal water oxygen atom of the solvent chain, O(W2), is simultaneously a hydrogen bond donor and a hydrogen bond acceptor with the atoms O(23) and O(14) respectively, where the latter belong to two ouabain molecules related by a unit cell translation in the z-direction (see Figure 1.12). O(W2) is also hydrogen bonded to O(W1). O(W1) exhibits the close contact  $O(W1) \cdots O(2')$  ( $2.94 \text{ \AA}$ ), as well as a third close contact with O(S2),  $(O(S2) \cdots O(W1))$ ,  $2.44 \text{ \AA}$ , see Figure 1.11).

The oxygen atom O(S2) of the half-methanol molecule C(S2)-O(S2) (bond length,  $1.54(3) \text{ \AA}$ ), forms only one close contact with the oxygen atoms of the glycoside  $(O(S2) \cdots O(14))$ ,  $2.96(2) \text{ \AA}$ , see Figure 1.12, the molecule located closest to the center of the cell). The two carbon atoms of the half-methanol molecules are located in a hydrophobic pocket of the cell. The two solvent carbon atoms are separated by  $1.10(3) \text{ \AA}$  and the angles C(S1)-C(S2)-O(S2) and C(S2)-C(S1)-O(S1) are  $79(2)$  and  $131(2)^\circ$  respectively.

The position of the half-oxygen atom O(S1),  $1.62 \text{ \AA}$  from C(S1), is defined by its simultaneous participation as a hydrogen bond acceptor with H(03') and H(04') in two ouabain molecules related by the screw diad axis at  $1/4, 0, z$ . The fact that it forms two hydrogen bonds with hydroxyl substituents of separate ouabain molecules concurs with the observation that its thermal parameters



are smaller than those for the inner members of the solvent chain. The two hydrogen bonds to O(S1) are visible in the upper right hand corner of the enclosure in Figure 1.12.

Although the manner in which the crystals gain or lose solvent is not evident from either diagram, this packing does allow the crystals to have a variable solvent of crystallization. In particular, O(S1) should be able to have an occupational factor of 1.0. The residual disordering in the final difference Fourier in the region around O(W1) and to a lesser extent O(W2) could be due to a statistical water-methanol disordering. Trivedi (32) suggests that ouabain recrystallized from methanol:water occurs as the tetrahydrate whereas ouabain crystals obtained from ethanol:water have an extra  $\frac{1}{2}$  molecule of water of crystallization. The most likely explanation of this phenomenon is the ability of methanol but not ethanol to replace water in the crystal lattice.

#### 1.4 BIOLOGICAL SIGNIFICANCE OF THE STRUCTURE

Since the oxygen atoms O(3), O(14) and O(23) have been considered important if not necessary for the cardiotonic activity of the cardiac glycosides and more recently for the binding of the digitalis compounds to the  $[\text{Na}^+, \text{K}^+]\text{-ATPase}$  receptor (13,25,29), the oxygen-oxygen separations and their orientation with respect to the steroid nucleus may necessarily be restricted to a limited range of values in order for binding to occur. Because the crystal structures of ouabain and digitoxigenin showed dissimilar orientations of the lactone ring with respect to the 14-hydroxyl substituent, the O(3)···O(14), O(3)···O(23) and O(14)···O(23) distances were calculated for each structure. These separations are 7.90, 13.48 and 6.32 Å for ouabain and 8.06, 13.21 and 5.32 Å for digitoxigenin.





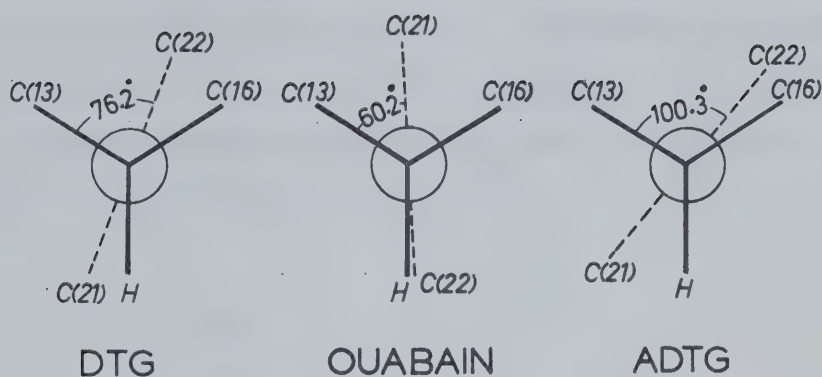


Figure 1.13 The orientation of the lactone ring with respect to the steroid nucleus observed in the crystal structures of digitoxigenin, ouabain and  $\Delta$ -8,14-anhydrodigitoxigenin.

One other group of natural products possesses a cardiotonic activity similar and equal to that observed for the cardiac glycosides. These compounds are the Erythrophleum alkaloids (25) and include cassaine, coumingine, ivorine and erythrophleine. Bonting, Hawkins and Canady (62)

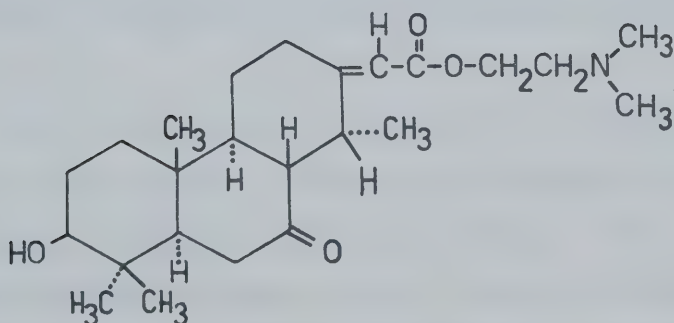


Figure 1.14 The chemical structure of cassaine.



found that a  $10^{-4}$ M concentration of cassaine inhibited 86% of the  $[\text{Na}^+, \text{K}^+]$ -ATPase activity for enzyme isolated from rabbit brain tissue but did not inhibit  $\text{Mg}^{++}$ -activated ATPase activity. The inhibition curves for ATPase activity as a function of inhibitor concentration were similar for ouabain, cassaine and erythrophleine. The  $I_{50}$  values for the three inhibitors were 1.1, 5.2 and  $0.3 \mu\text{M}$  respectively.

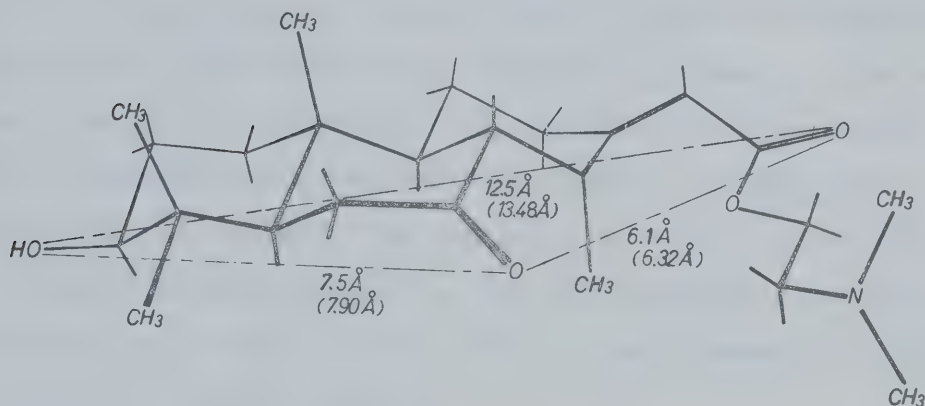


Figure 1.15 The 'active' conformation of the cassaine molecule showing the oxygen...oxygen separations. The analogous separations observed in ouabain tetrahydrate are included in parentheses.

Prentiss-Hall molecular models were constructed for ouabain, digitoxigenin and cassaine. Only when the conformation of the cassaine model was made to agree with that shown in Figure 1.15 did the spatial relationship between the ester carbonyl, 7-keto and 3-hydroxyl oxygen atoms mimic the spatial disposition of O(23), O(14) and O(3) in ouabain. The conformation of cassaine could not be adjusted so that the disposition of the same three oxygen atoms would simultaneously agree with the



analogous oxygen-oxygen separations observed in the crystal structure of digitoxigenin, (see Figure 1.5). The greatly reduced cardiotonic activity of the  $\Delta$ -8,14-anhydrodigitoxigenin derivative which has an  $O(23)\cdots O(3)$  separation of  $13.36 \text{ \AA}$ , suggests that the 14-hydroxyl is involved in the formation of the enzyme-inhibitor complex, and strengthens the hypothesis that the active conformation of the cardiac glycosides is similar to the conformation observed in the crystal structure of ouabain tetrahydrate.

Clarke et al (63) tested a number of synthetic 7-dehydro cassaine derivatives, lacking the 4-,10- or 14-methyl substituents, on isolated rabbit hearts and spontaneously respiring dogs. A positive inotropic effect, somewhat reduced with respect to cassaine, was observed. This finding does not contradict the supposition that the 7-keto substituent in cassaine (or the 14-hydroxyl in ouabain) is involved in the complementary binding of the cardiotonic drug to a multicomponent binding site on the  $[Na^+, K^+]$ -ATPase receptor.

Clarke et al (63) observed that the synthetic 7-dehydro cassaine derivative containing the ester side chain *cis* with respect to the C(13)-C(14) bond was equally potent as the *trans*-isomer. However in both derivatives the 3-hydroxyl...ester carbonyl oxygen separation is identical. The  $3\beta$ -hydroxyl can be replaced by a  $3\alpha$ -hydroxyl without loss of drug activity. Replacement of the  $3\beta$ -hydroxyl by an ethylenedisulfonyl moiety increased the drug activity whereas a large decrease in drug activity was observed when the 3-hydroxyl was replaced by hydrogen. The  $3\alpha$ -hydroxyl...ester carbonyl oxygen separation can be made identical to the  $3\beta$ -hydroxyl...ester oxygen separation by giving the A-ring of the phenanthrene nucleus a boat conformation. This is not true however when the same transformation is applied to ouabain or digitoxigenin.



The hypothesis that the active conformation of the cardiac glycoside molecule, as it binds to the  $[\text{Na}^+, \text{K}^+]$ -ATPase receptor, corresponds to the conformation observed in the structure of ouabain tetrahydrate is attractive. However it should be accepted with some reservation in view of the complexity of the  $[\text{Na}^+, \text{K}^+]$ -ATPase system. Lindemeyer and Schwartz (64) suggest that binding of ouabain to calf-brain membranial  $[\text{Na}^+, \text{K}^+]$ -ATPase is accompanied by a change in the conformation of the enzyme. The allosteric nature of the ouabain inhibition of this enzyme is also supported by Charnock, Cook and Opit (27).

Finally, no specific mention has been made about the role of the steroid nucleus in the formation of an enzyme-inhibitor complex. The cardiac glycosides are characterized by a total absence of axial  $\alpha$ -hydroxyl substituents attached to the steroid nucleus. Therefore, a hydrophobic interaction between this surface and the enzyme is certainly a distinct possibility.

In view of the equality of the O(23)···O(3) separation in ouabain with the ester carbonyl oxygen-O(3) separation in cassaine, the hypothesis of Wilson et al (29) that the enzyme and lactone ring interact by a  $\pi$ -bonding interaction is less likely. It is more probable that the carbonyl oxygen acts as a hydrogen bond acceptor as was initially proposed by Repke (13). Its ability to do so is demonstrated in this crystal structure.





## CHAPTER 2

## THE CRYSTAL AND MOLECULAR STRUCTURE OF GUTIEROLIDE

2.1 INTRODUCTION

The use of X-ray crystallography for the elucidation of molecular structures of natural products has increased greatly during the last decade due to the availability of automated diffractometers and high speed computers. The requirement of only a very minute quantity of crystalline material makes this technique the most powerful available, particularly when the compound is isolated in trace amounts.

In recent years a small number of halogenated terpenes have been found in both terrestrial and marine flora. These compounds are listed in Table 2.1 with their chemical formulae and the species from which they were isolated. The occurrence of four different chlorine containing terpenes among several members of the Compositae is not surprising in that evidence of polyacetylenic epoxides, chlorohydrins and acetates in that family has been well documented (65).

Gutierolide was first isolated in the laboratory of R.W. Doskotch from the alcoholic percolate of the above ground parts of Gutierrezia dracunuloides (DC) Blake. The amount of compound isolated was too little to permit a classical structural determination and therefore only a preliminary spectral and chemical analysis were performed (42). This analysis indicated that the molecule contained a covalently bound chlorine atom. The infra-red spectrum in chloroform indicated the presence of lactone carbonyl and hydroxyl substituents. A positive Kedde test together with an ultraviolet absorption band at 210 nm. suggested the



presence of a butenolide moiety.

The terpene formed several well shaped crystals by evaporation of a methanolic solution. This and the presence of the chlorine atom make a crystal structure determination a highly feasible project.

Table 2.1

Naturally Occurring Halogenated Terpenes

|                        |                         |   |      |
|------------------------|-------------------------|---|------|
| Eupachlorin<br>Acetate | $C_{22}H_{27}O_8Cl$     | Eupatorium rotundi-<br>folium L. (Compositae)             | (66) |
| Eupachlorin            | $C_{20}H_{25}O_7Cl$     | Eupatorium rotundi-<br>folium L.                          | (66) |
| Eupachloroxin          | $C_{20}H_{25}O_8Cl$     | Eupatorium rotundi-<br>folium L.                          | (66) |
| Centaurepensin         | $C_{19}H_{24}O_7Cl_2$   | Centaurea repens L.<br>( 'Russian Knapweed' )             | (67) |
| Johnstonol             | $C_{15}H_{21}O_3Br_2Cl$ | Laurencia Johnstonii<br>(red alga)                        | (68) |
| Pacifenol              | $C_{15}H_{20}O_2Br_2Cl$ | Laurencia Pacifica  | (69) |
| Gutierolide            | $C_{21}H_{31}O_5Cl$     | Gutierrezia dracunul-<br>oides (DC) Blake<br>(Compositae) | (42) |

## 2.2 EXPERIMENTAL

Several well formed colorless prisms of gutierolide were supplied by R.W. Doskotch. The isolation and purification of this compound plus a preliminary chemical and spectral characterization were performed in that laboratory and are communicated in (42). No attempt was made to recrystallize this material or to measure the crystal density due to the small number of crystals available.



Table 2.2

## Gutierolide Physical and Crystallographic Data

|   |  |
|---|--|
| Formula                                 | $C_{21}H_{31}O_5Cl$  |
| Molecular Weight                        | 398.9  |
| Melting point                           | 207-209°C  |
| Optical rotation                        | $[\alpha]_D^{21} = -103^\circ$ (methanol)                        |
| Space group                             | $P2_1^2 1^2 1$   |
| Cell dimensions                         | $a = 15.217(4) \text{ \AA}$<br>$b = 17.397(3)$<br>$c = 7.353(2)$ |
| Volume                                  | $V = 1946.1 \text{ \AA}^3$                                       |
| Cell content                            | $z = 4$ molecules/cell   |
| Absorption coefficient                  | $\mu = 19.9 \text{ cm}^{-1}$ (Cu $K\alpha$ )                     |
| Calculated density                      | $\rho_c = 1.36 \text{ g/cm}^3$                                   |
| No. of independent reflections measured | 1882   |
| No. of independent reflections observed | 1555   |
| Wavelength used in data collection      | Cu $K\alpha$   |
| Range of reciprocal space explored      | $3^\circ < 2\theta < 129^\circ$                                  |
| Number of parameters                    | 368  |
| Final residual                          | $R = 0.033$  |
| Final weighted residual                 | $R_w = 0.035$  |



Unit cell data and physical constants are listed in Table 2.2. Preliminary diffraction photographs of the  $h0l$  and  $hk0$  zones displayed mm symmetry with the odd-ordered reflections of the three principal axes systematically unobserved. Weissenberg photographs of the  $hkl$  to  $hk4$  zones showed mm symmetry with no systematic absences. From this evidence the space group was deduced as  $P2_1^2 2_1^2 2_1^2$ . Accurate cell dimensions were obtained by the method of Busing and Levy (33) using the accurately determined  $2\theta$ ,  $\omega$ ,  $\chi$  and  $\phi$  coordinates of 12 reflections.

The intensity data were collected for a tabular crystal  $0.2 \times 0.15 \times 0.3$  mm ( $a \times b \times c$ ) using the FACS-1 diffractometer and graphite monochromatized Cu  $K\alpha$  radiation at a take-off angle of  $5^\circ$ . Reflections were scanned in the continuous  $\theta/2\theta$  moving crystal/moving detector mode at a rate of  $2^\circ 2\theta/\text{min.}$ , using a standard reflection width of  $1.5^\circ 2\theta$ , modified for the dispersion of the  $\alpha_1 \alpha_2$  doublet. Stationary backgrounds were counted for 10 seconds at the  $2\theta$  boundaries of each reflection. The crystal was monitored for decomposition by the collection of three standard reflections once every 33 intensity measurements. During the data collection the average decrease in the intensities of the standard reflections was less than 1% of their initial values.

The intensity data were corrected for decomposition, Lorentz and polarization effects (34). A total of 1555 reflections had intensities  $I > 3\sigma(I)$  and were considered observed. An observational weight  $w$  was calculated for each reflection using the formula given in the Preface. The overall temperature factor,  $3.59 \text{ \AA}^2$ , and scale, 0.7839, were estimated from a Wilson plot (36) which is shown in Figure 2.1. No absorption corrections were done due to the low absorption coefficient  $\mu = 19.9 \text{ cm}^{-1}$  and the small equidimensional shape of the crystal.





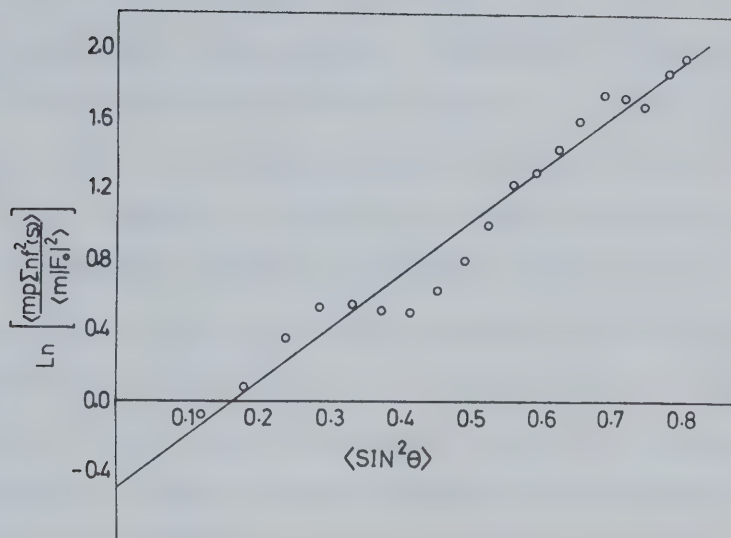


Figure 2.1 The gutierolide Wilson plot. The atomic formfactors  $f(s)$  are summed for all atoms in the unit cell;  $m$  is the reflection multiplicity,  $p$  is a factor to account for systematically absent reflections.

A Patterson synthesis computed with  $1/Lp$  sharpened coefficients showed a large vector peak on each of the three Harker sections. These three peaks formed a self-consistent set expected for an atom in the space group  $P2_12_12_1$ . These peaks were tentatively identified to be the chlorine-chlorine vectors and gave the coordinates  $x/a = 0.000$ ,  $y/b = -0.033$  and  $z/c = 0.568$ . A minimum symmetry function (70) calculated with this chlorine atom position using the  $1/Lp$  sharpened Patterson synthesis showed two peaks which were interpreted as part of an atom chain bonded to the



chlorine atom. An extension of the chain to additional atoms could not be made at this stage. The extra symmetry in this minimum function resulting from the pseudo-special coordinates of the chlorine atom prevented the unambiguous identification of additional structural fragments.

A structure factor calculation based on one chlorine and two carbon atoms yielded the residual  $R=0.55$ . Heavy-atom Fourier syntheses with coefficients weighted according to the method of Sim (71) produced a chemically plausible structure containing 18 additional atoms. However attempted refinement of the positional parameters for this 21 atom model did not reduce the residual below 0.42. An examination of the molecular packing showed several close intermolecular contacts and large gaps in the cell. A difference Fourier synthesis computed from the  $R=0.42$  parameters showed no additional atoms.

To determine which atoms were a part of the true structure, only atomic positions common to both the minimum function and the Fourier maps and forming a chemically plausible structure were considered. Seven atomic positions satisfied these conditions - the three atom fragment  $C(3)-C(3)-C(2)$  and a four atom chain  $C(17)-O(17)-C(18)-O(18)$ . A structure factor calculation based on the chlorine atom plus six carbon atoms produced the residual  $R=0.50$ . At this stage of the structure solution, the use of Fourier techniques was discontinued.

Normalized structure factors  $|E_{hkl}|$  were calculated by the relationship

$$|E_{hkl}| = \left\{ \frac{k(\sin^2 \theta)}{\sum_{i=1, N} n f_i^2(s)} \right\}^{\frac{1}{2}} \cdot |F_{hkl}|$$



where  $k(\sin^2 \theta)$  is the normalization curve which places  $|F_{hkl}|$  on absolute scale for atoms at rest. A comparison of the  $|E|$  statistics for gutierolide with the theoretical values for centric and acentric space groups (37) is given in Table 2.3.

Table 2.3

## E-Statistics for Gutierolide

|                             | Observed | Centrosymmetric | Noncentrosymmetric |
|-----------------------------|----------|-----------------|--------------------|
| $\langle  E  \rangle$       | 0.862    | 0.798           | 0.886              |
| $\langle  E ^2 \rangle$     | 0.992    | 1.000           | 1.000              |
| $\langle  E^2 - 1  \rangle$ | 0.790    | 0.968           | 0.736              |
| $ E  > 3.0$                 | 0.00%    | 0.27%           | 0.01%              |
| $ E  > 2.5$                 | 0.69     | 1.24            | 0.19               |
| $ E  > 2.0$                 | 2.23     | 4.55            | 1.83               |
| $ E  > 1.0$                 | 35.23    | 31.73           | 36.79              |

A total of 221 reflections were found with normalized structure factors  $|E|$  greater than 1.50. A listing was prepared giving the members of each reflection triplet satisfying the sigma-2 equation (4) and sorted in decending values of  $|E|$ . From the partial structure based on seven atoms, 69 reflections with  $|E| > 1.72$  and  $(|F_c|/0.7839 |F_o|) > 0.4$  were used as starting phases for the phase extension program based on the tangent formula (5). These phases were kept fixed during the phase refinement process. The following restrictions were applied before a new reflection phase was accepted.



- (i) The reflection must be determined in at least three independent triplets.
- (ii)  $|E_c|$  must be greater than 0.4.
- (iii) Reflections showing large oscillations in phase angle must be removed from the refinement for one iteration step and redetermined in the next step.

Two cycles of phase refinement were performed with E-threshold values of 1.75 and 1.51 which resulted in  $R_k$  values of 0.32 and 0.25. An E-map phased on 197 of a possible 201 reflections with  $|E| > 1.51$  showed sixteen promising positions which were part of a three ring fused skeleton shown in heavy outline in Figure 2.2.

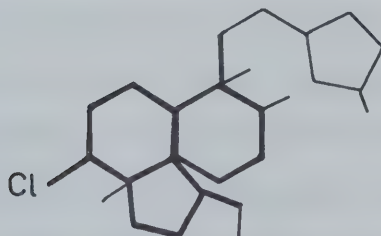


Figure 2.2 The structural fragment indicated by the first E-map.

A structure factor calculation based on one chlorine and fifteen carbon atoms gave the residual  $R=0.41$ . Two cycles of tangent refinement using 82 phased reflections based on this fragment with  $|E| > 1.725$  and  $|F_c/kF_o| > 0.5$  resulted in  $R_k$  values of 0.23 and 0.22 for E-threshold values of 1.75 and 1.51. A second E-map computed from 216 phased reflections showed a butenolide moiety plus two atoms attached to the previous skeletal fragment.





A structure factor calculation based on 24 atoms produced the residual  $R=0.30$  and a difference Fourier was computed which established the positions of three additional atoms. The initial residual based on the molecular formula  $C_{25}OCl$  was 0.23. The positional and isotropic thermal parameters of each atom were refined in four cycles of least-squares using unit weights and the block-diagonal approximation, decreasing the residual to 0.13. The quantity minimized was  $\sum w ||F_o| - |F_c||^2$ .

A difference Fourier synthesis at this residual showed that four of the input carbon atoms were oxygen atoms. The hydrogen atoms bonded to methyl carbons appeared as maxima of 0.3 to 0.5  $e/A^3$ . The other hydrogen atoms in the map were too ill-defined to permit an accurate determination of their positional parameters. The identity of the oxygen atoms was confirmed by a calculation of interatomic distances. The final molecular formula was established as  $C_{21}H_{31}O_5Cl$ .

To facilitate the refinement, the positions of all remaining hydrogen atoms attached to carbon were calculated by the method of Cradwick (38) assuming the appropriate bonding geometry and a carbon-hydrogen distance of 1.04 Å. Each hydrogen atom was assigned the isotropic thermal parameter of the covalently bonded carbon atom and included in the structure factor calculation. Refinement of positional and isotropic thermal parameters of all non-hydrogen atoms for three cycles of least-squares changed the residual from 0.120 to 0.098. The convergence of all parameter shifts was sufficient to warrant changing to an anisotropic thermal description for the non-hydrogen atoms. The thermal parameters of the hydrogen atoms were set to the final isotropic thermal parameters of the attached carbon atoms and kept constant for the remainder of the refinement.



The reflections were then assigned the associated observational weights. Two cycles of refinement decreased the residual to 0.049. The position of the hydroxyl hydrogen atom was determined from a difference Fourier to be approximately  $0.7 \text{ \AA}$  from O(4) and  $1.9 \text{ \AA}$  from O(18) in the same molecule. The refinement was continued for 4 cycles of least-squares allowing the positional parameters of the hydrogen atoms to refine. During this process several reflections with very large intensities were found to have large individual discrepancies of the type  $|F_c| > |F_o|$  which indicated a systematic error in  $|F_o|$  due to secondary extinction and/or counter paralysis. To minimize the effects of these errors on the refinement, each reflection was given a modified weight  $w$  such that  $w ||F_o| - |F_c||^2 = 0.5$ .

The form factor for chlorine was corrected for the imaginary component of anomalous dispersion using the value  $\Delta f_{Cl}'' = 0.7$  electrons (10). In order to determine the correct optic hand of the molecule, a second set of atomic parameters was created by inverting the first set of atomic coordinates through the origin of the unit cell. Two sets of parameters were refined independently in the previously described manner until all parameter shifts were less than 0.1 of the corresponding e.s.d. The final values of the weighted and unweighted residuals  $R_w$  and  $R$  for the two enantiomorphs are listed below:

|       | Parameter<br>set A | Parameter<br>set A' | Ratio<br>A'/A |
|-------|--------------------|---------------------|---------------|
| R     | 0.033              | 0.044               | 1.33          |
| $R_w$ | 0.035              | 0.049               | 1.40          |



Interpolation of the R-factor ratios on the Tables of Hamilton (72) indicated each to be significant at the 99.5% confidence level so that the absolute configuration of the molecule is defined by parameter set A.

A difference Fourier computed from the final set of parameters exhibited a residual difference electron density between 0.10 and -0.10  $\text{e}/\text{\AA}^3$  for all regions of the unit cell except in the vicinity of the chlorine position where the extremes were +0.15 and -0.20  $\text{e}/\text{\AA}^3$ .

The final positional parameters of all atoms are listed in Tables 2.4 and 2.5 with the e.s.d. of the least significant digit in parentheses. The atom designation of the skeletal atoms is consistent with standard terpene nomenclature with the exception of the methoxy carbon which is designated as C(21). Hydrogen atoms have been designated by multiplying the number of the bonded carbon atom by 10 and adding 1, 2 or 3. The final thermal parameters of the anisotropic atoms are listed in Table 2.6 as mean square amplitudes of vibration in  $\text{\AA}^2$ .

The final value of the scale factor required to bring the observed structure factors onto absolute scale is 0.8035. The observed and calculated structure factors are listed in Table 2.7 on the absolute scale  $\times 10$ . Reflections which were down weighted in the terminal stages of refinement to minimize the effects of extinction and/or counter paralysis are designated with a dagger ( $\dagger$ ). Unobserved reflections are designated with an asterisk (\*).



Table 2.4

The positional parameters ( $\times 10^5$ ) of the non-hydrogen atoms of gutierolide.

| ATOM         | x/a       | y/b       | z/c       |
|--------------|-----------|-----------|-----------|
| C $\ell$ (3) | 100617(6) | 103310(4) | 43153(14) |
| C(1)         | 80504(20) | 89911(16) | 61935(42) |
| C(2)         | 88888(20) | 94590(16) | 62075(44) |
| C(3)         | 90731(18) | 97494(14) | 43244(44) |
| C(4)         | 91606(17) | 91069(14) | 29328(41) |
| C(5)         | 83813(16) | 85101(14) | 29796(40) |
| C(6)         | 75783(18) | 87947(15) | 19206(42) |
| C(7)         | 68391(18) | 82053(16) | 19242(44) |
| C(8)         | 65056(17) | 80213(15) | 38283(42) |
| C(9)         | 72656(17) | 77516(14) | 50955(40) |
| C(10)        | 81130(17) | 82773(14) | 49569(40) |
| C(11)        | 74434(18) | 68974(14) | 45428(41) |
| C(12)        | 82114(19) | 64876(15) | 54421(44) |
| C(13)        | 81371(19) | 56248(15) | 53366(41) |
| C(14)        | 74508(21) | 51834(16) | 50304(48) |
| C(15)        | 77087(23) | 43783(16) | 52018(46) |
| C(16)        | 89081(21) | 51252(16) | 57186(55) |
| C(17)        | 99696(19) | 85977(15) | 32426(46) |
| C(18)        | 88747(18) | 78356(14) | 20571(40) |
| C(19)        | 59193(19) | 86712(16) | 45403(47) |
| C(20)        | 69534(20) | 77201(17) | 70861(46) |
| C(21)        | 95252(22) | 74528(20) | -7175(47) |
| O(4)         | 92050(13) | 94838(10) | 12129(29) |
| O(15)        | 72698(19) | 38002(12) | 50492(37) |
| O(16)        | 85641(15) | 43481(11) | 56035(40) |
| O(17)        | 96964(12) | 78177(10) | 29380(30) |
| O(18)        | 89722(12) | 79993(10) | 1792(26)  |





Table 2.5

Positional and thermal parameters ( $\times 10^4$ )  
for the hydrogen atoms of gutierolide.

| ATOM   | x/a       | y/b       | z/c       | $U_{iso}$ |
|--------|-----------|-----------|-----------|-----------|
| H(11)  | 7932(16)  | 8808(13)  | 7361(35)  | 372       |
| H(12)  | 7566(16)  | 9294(13)  | 5929(37)  | 372       |
| H(21)  | 9367(16)  | 9173(14)  | 6639(39)  | 414       |
| H(22)  | 8848(16)  | 9901(14)  | 7161(36)  | 414       |
| H(31)  | 8606(15)  | 10074(14) | 3988(36)  | 390       |
| H(61)  | 7749(15)  | 8941(13)  | 741(35)   | 342       |
| H(62)  | 7412(15)  | 9255(13)  | 2574(34)  | 342       |
| H(71)  | 7029(16)  | 7732(13)  | 1306(37)  | 372       |
| H(72)  | 6334(16)  | 8417(13)  | 1235(37)  | 372       |
| H(81)  | 6073(17)  | 7580(14)  | 3664(37)  | 410       |
| H(101) | 8605(15)  | 8005(12)  | 5405(33)  | 295       |
| H(111) | 6887(15)  | 6643(13)  | 4921(36)  | 332       |
| H(112) | 7475(15)  | 6848(13)  | 3260(36)  | 332       |
| H(121) | 8777(17)  | 6638(13)  | 4913(36)  | 394       |
| H(122) | 8222(16)  | 6600(13)  | 6806(39)  | 394       |
| H(141) | 6865(17)  | 5339(15)  | 4717(40)  | 486       |
| H(161) | 9115(18)  | 5240(16)  | 7122(41)  | 559       |
| H(162) | 9346(18)  | 5174(16)  | 4882(41)  | 559       |
| H(171) | 10426(16) | 8723(13)  | 2404(35)  | 389       |
| H(172) | 10214(16) | 8600(13)  | 4524(37)  | 389       |
| H(181) | 8622(16)  | 7307(13)  | 2208(34)  | 351       |
| H(191) | 6225(18)  | 9158(14)  | 4852(38)  | 461       |
| H(192) | 5451(17)  | 8796(14)  | 3536(39)  | 461       |
| H(193) | 5620(17)  | 8518(14)  | 5641(39)  | 461       |
| H(201) | 7454(17)  | 7559(14)  | 7869(38)  | 431       |
| H(202) | 6731(16)  | 8216(14)  | 7530(37)  | 431       |
| H(203) | 6399(17)  | 7395(14)  | 7134(37)  | 431       |
| H(211) | 9191(18)  | 6889(16)  | -543(41)  | 554       |
| H(212) | 10074(19) | 7534(16)  | -396(43)  | 554       |
| H(213) | 9537(18)  | 7589(15)  | -1983(42) | 554       |
| H(04)  | 9288(17)  | 9115(14)  | 324(39)   | 500       |



Table 2.6

The parameters describing the anisotropic thermal vibration of the atoms listed in Table 2.4. All quantities are multiplied by  $10^4$ .

| ATOM  | $U_{11}^*$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{23}$ | $U_{13}$ |
|-------|------------|----------|----------|----------|----------|----------|
| C(3)  | 239(2)     | 181(2)   | 337(3)   | -85(2)   | -19(2)   | -11(2)   |
| C(1)  | 198(7)     | 156(6)   | 150(7)   | -8(6)    | -17(6)   | 12(6)    |
| C(2)  | 187(7)     | 143(6)   | 201(8)   | -4(6)    | -43(6)   | -31(7)   |
| C(3)  | 146(6)     | 103(5)   | 237(8)   | -10(5)   | -4(6)    | -20(7)   |
| C(4)  | 139(6)     | 121(6)   | 161(7)   | -6(5)    | 14(6)    | 4(6)     |
| C(5)  | 120(6)     | 98(5)    | 145(6)   | 5(5)     | 10(5)    | 1(5)     |
| C(6)  | 144(6)     | 139(6)   | 149(7)   | 23(5)    | 21(6)    | 3(6)     |
| C(7)  | 128(6)     | 168(6)   | 164(7)   | 7(5)     | 0(6)     | -27(6)   |
| C(8)  | 117(6)     | 136(6)   | 187(7)   | -11(5)   | 1(6)     | -4(6)    |
| C(9)  | 129(6)     | 115(6)   | 149(7)   | -3(5)    | 2(5)     | 6(6)     |
| C(10) | 128(5)     | 116(5)   | 139(7)   | 0(5)     | 1(5)     | -11(6)   |
| C(11) | 149(6)     | 106(5)   | 168(7)   | -15(5)   | 0(6)     | -2(6)    |
| C(12) | 165(6)     | 116(6)   | 200(8)   | -12(5)   | 2(6)     | -10(7)   |
| C(13) | 176(6)     | 125(6)   | 152(7)   | 3(5)     | 9(6)     | 7(6)     |
| C(14) | 205(7)     | 141(6)   | 253(9)   | -17(6)   | 19(6)    | -49(8)   |
| C(15) | 297(9)     | 133(6)   | 183(8)   | -22(6)   | 0(6)     | -1(8)    |
| C(16) | 197(8)     | 136(6)   | 346(11)  | 16(6)    | 11(8)    | 33(9)    |
| C(17) | 132(6)     | 147(6)   | 244(9)   | 5(6)     | -16(6)   | -8(7)    |
| C(18) | 135(6)     | 119(6)   | 156(7)   | 10(5)    | 2(6)     | 14(6)    |
| C(19) | 153(7)     | 183(7)   | 237(9)   | 39(6)    | 15(7)    | 28(7)    |
| C(20) | 196(7)     | 186(7)   | 170(8)   | -28(6)   | 3(7)     | 34(7)    |
| C(21) | 227(8)     | 265(9)   | 182(8)   | 59(7)    | -50(8)   | 34(8)    |
| O(4)  | 199(5)     | 141(4)   | 192(5)   | -14(4)   | 39(4)    | 39(5)    |
| O(15) | 449(8)     | 140(5)   | 303(8)   | -80(6)   | 4(5)     | -52(7)   |
| O(16) | 279(6)     | 123(5)   | 392(8)   | 37(5)    | 18(5)    | 14(7)    |
| O(17) | 140(4)     | 124(4)   | 222(6)   | 28(3)    | 3(4)     | -17(1)   |
| O(18) | 171(4)     | 154(4)   | 151(5)   | 23(4)    | -8(4)    | 28(4)    |

\* These coefficients are defined in the Preface.





Table 2.7

The observed and calculated structure factor amplitudes, on the absolute scale  $\times 10$  for gutierólíde. The unobserved reflections are indicated by an asterisk (\*). Reflections which were assigned a modified weight during the late stages of the refinement are indicated by a dagger (†).

[illegible]

Continued...









## 2.3 RESULTS AND DISCUSSION

### 2.3.1 Absolute Configuration and Classification of the Terpene

The molecular structure of gutierolide is depicted in the correct absolute configuration in Figure 2.3. The configuration of the asymmetric carbon atoms according to the R, S-designation of Cahn, Ingold and Prelog (73) is: C(3)-R, C(4)-R, C(5)-S, C(8)-S, C(9)-R, C(10)-S, C(18)-S.

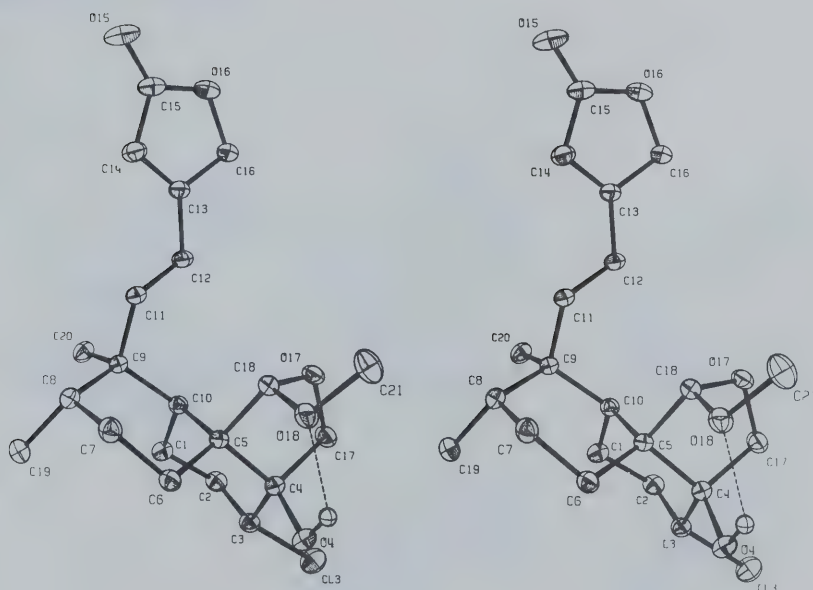


Figure 2.3 The molecular structure of gutierolide. The anisotropic thermal ellipsoids of the non-hydrogen atoms are scaled to include 35% probability. The intramolecular hydrogen bond is shown by a dashed line.



Gutierolide belongs to the kolavane skeletal class of diterpenes and shows a marked resemblance to the diterpenes solidago lactol (74), solidago epoxylactol (74), olearin (75), and hautriwaic acid (76) whose structural formulae are represented in Figure 2.4. The ring junctions in gutierolide are A/B cis and A/C cis. Both six membered rings exhibit chair conformations which are somewhat distorted due to strain at the ring junctions. The stereochemistry of the ring fusions in the related diterpenes has been determined only for hautriwaic acid where chemical evidence suggests that the A/B ring fusion is trans.

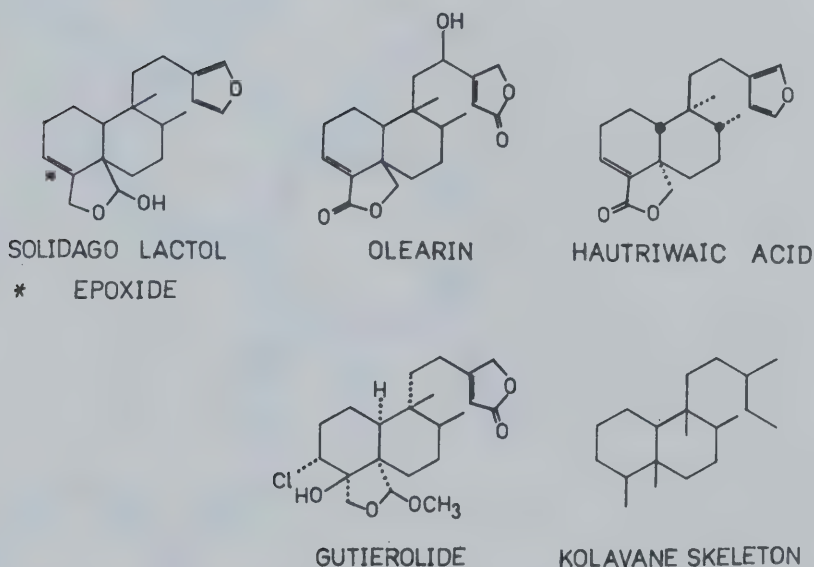


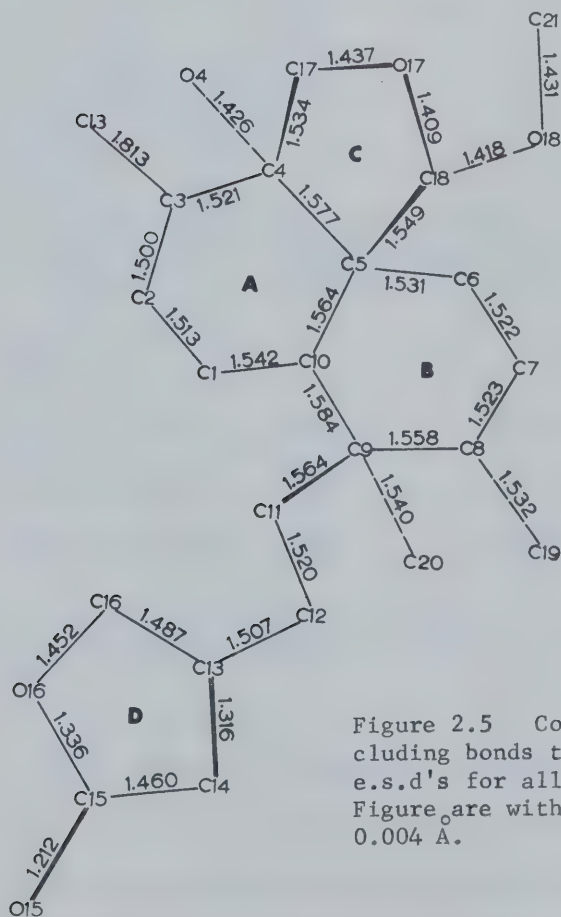
Figure 2.4 The structural formulae of gutierolide and several other diterpenoid lactones belonging to the kolavane skeletal class of diterpenes.



The molecular structure of gutierolide substantiates the preliminary spectral and chemical properties observed by Dосkotch (42). However, the mass spectrum did not show a parent ion, so that the initially proposed chemical formula of  $C_{20}H_{29}O_5Cl$  did not account for an additional 14 mass units ( $CH_2$ ).

### 2.3.2 Molecular Geometry

The covalent bond distances and interbond angles excluding hydrogen atoms are illustrated in Figures 2.5 and 2.6 respectively.







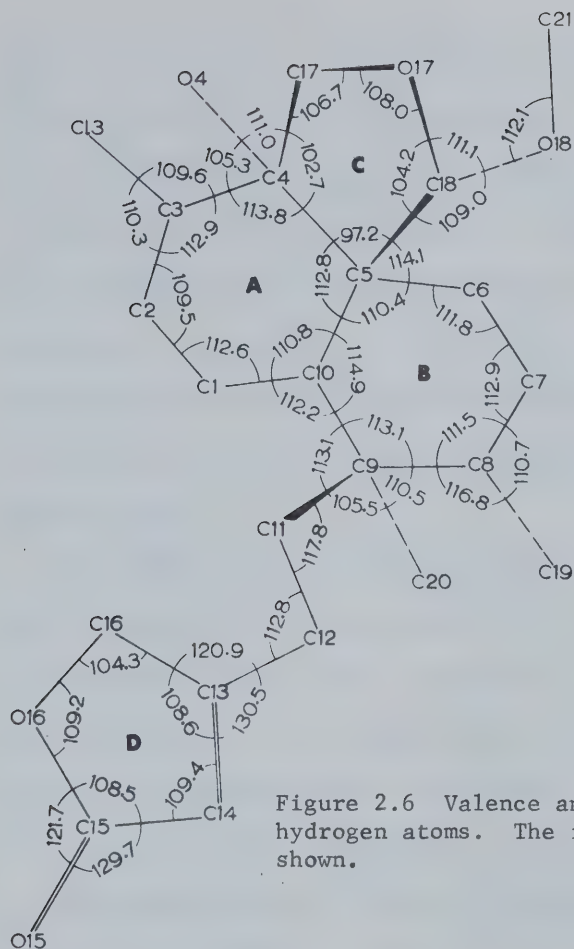


Figure 2.6 Valence angles excluding bonds to hydrogen atoms. The following angles are not shown.

|                 |        |                  |        |
|-----------------|--------|------------------|--------|
| C(3)-C(4)-C(17) | 113.3° | O(4) -C(4)-C(5)  | 111.0° |
| C(4)-C(5)-C(6)  | 112.1  | C(10)-C(5)-C(18) | 109.7  |
| C(8)-C(9)-C(10) | 113.1  | C(10)-C(9)-C(20) | 109.4  |

The e.s.d's for valence bond angles excluding hydrogen atoms range from 0.2 to 0.3°.

The Cl(3)-C(3) distance of 1.813(3) Å is significantly longer than the average value of 1.767(5) Å reported by Sutton (6) for 31 compounds containing Cl-C(sp<sup>3</sup>) covalent bonds. The Cl(3)-C(3) bond length was corrected for 'riding motion' assuming the chlorine



atom to ride on C(3). This yielded a value of 1.829 Å. Long C(sp<sup>3</sup>)-C<sup>o</sup>l bonds have been reported for photoaldrin (77) (1.804(4) Å), α-chlorotropene monohydrate (78) (1.839(13), 1.830(8) Å) and 1-chloro-tetra-O-acetyl-α-D-glucopyranose (79) (1.811(10) Å). In each of these examples, chlorine is attached to a highly substituted ring structure. In contrast, the literature value reported by Sutton was obtained for simple halogenated paraffins mainly by the use of microwave and electron diffraction techniques for molecules in the gaseous state.

The C(sp<sup>3</sup>)-C(sp<sup>3</sup>) bond distances range from 1.584(4) Å to 1.500(4) Å with a weighted mean of 1.543(6) Å. The variation of the carbon-carbon distances about this mean is significant with the distance increasing with the number of non-hydrogen substituents to each of the bonded atoms. This phenomenon was also observed and discussed for the structure of ouabain tetrahydrate in Chapter 1.

The conformations of ring bonds containing two or three non-hydrogen substituents to each of the atoms in the bond are shown in Figure 2.7. Torsion angles about ring bonds not contained in this Figure are listed in Table 2.8 following the sign convention of Klyne and Prelog (44).

Because the Van der Waals radius of methylene (2.0 Å, (45)) is larger than hydrogen, those bonds containing a large number of methylene substituents may be lengthened due to repulsions between non-bonded methylene substituents which are gauche with respect to each other. This repulsive interaction increases as the CH<sub>2</sub>-C-C-CH<sub>2</sub> torsion angle approaches 0°. An examination of Figure 2.7 shows that whereas the substituents to the two longest C-C bonds (C(4)-C(5),



1.577 Å; C(9)-C(10), 1.584 Å) exhibit a semi-eclipsed conformation, the substituents to the C(3)-C(4) bond (1.521 Å) have a more staggered conformation. Gauche interactions involving Cl(3) or O(4) will also be smaller since chlorine and oxygen each have a smaller Van der Waals radius than methylene.

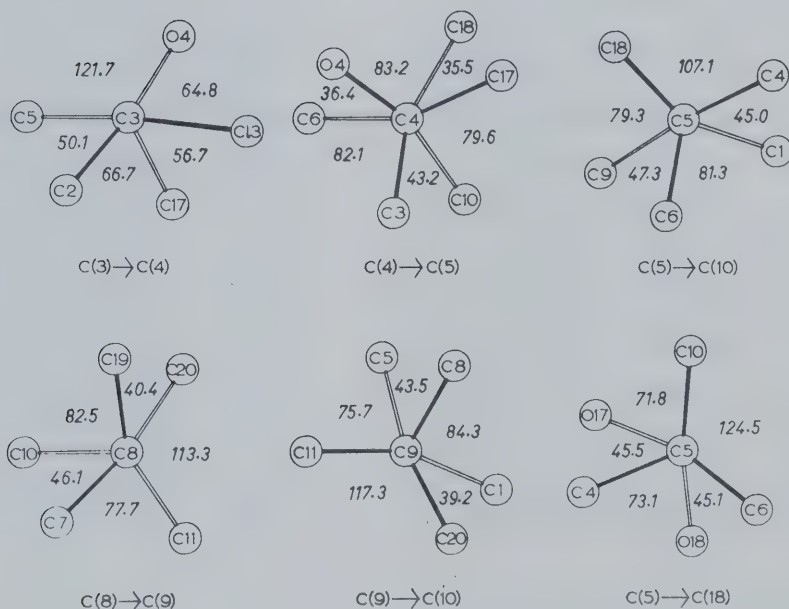


Figure 2.7 Newman projections down ring bonds containing two or three non-hydrogen substituents to each atom of the bond. Bonds which are shaded in are to the uppermost atom in the projection.



Table 2.8

Torsion angles about ring bonds  
not depicted in Figure 2.7.

| A-B-C-D                | $\tau$ | A-B-C-D                 | $\tau$ |
|------------------------|--------|-------------------------|--------|
| C(10)-C(1)-C(2) -C(3)  | +62.7  | C(6) -C(7) -C(8) -C(19) | +76.4  |
| C(1) -C(2)-C(3) -C(4)  | -58.4  | C(8) -C(9) -C(11)-C(12) | +159.9 |
| C(1) -C(2)-C(3) -C(3)  | +178.6 | C(10)-C(9) -C(11)-C(12) | +51.2  |
| C(3) -C(4)-C(17)-O(17) | +138.5 | C(20)-C(9) -C(11)-C(12) | -68.2  |
| O(4) -C(4)-C(17)-O(17) | -76.8  | C(9) -C(11)-C(12)-C(13) | +20.1  |
| C(5) -C(4)-C(17)-O(17) | +15.3  | C(11)-C(12)-C(13)-C(14) | +173.0 |
| C(4) -C(5)-C(6) -C(7)  | +178.3 | C(4) -C(17)-O(17)-C(18) | +14.2  |
| C(10)-C(5)-C(6) -C(7)  | -55.0  | C(17)-O(17)-C(18)-C(5)  | -38.8  |
| C(18)-C(5)-C(6) -C(7)  | +69.1  | C(17)-O(17)-C(18)-O(18) | +78.3  |
| C(5) -C(6)-C(7) -C(8)  | +61.0  | C(5) -C(18)-O(18)-C(21) | +171.1 |
| C(6) -C(7)-C(8) -C(9)  | -55.4  | O(17)-C(18)-O(18)-C(21) | -57.9  |

A positive torsion angle ( $\tau$ ) indicates that a clockwise rotation of the uppermost (A-B) bond about the B-C axis will bring A-B and C-D into coincidence (44).





Table 2.9

Intramolecular hydrogen...hydrogen  
contacts less than 2.4 Å.\*

| Atom A | Atom B | Distance            | Atom A | Atom B | Distance            |
|--------|--------|---------------------|--------|--------|---------------------|
| H(11)  | H(21)  | 2.34 Å <sup>o</sup> | H(71)  | H(82)  | 2.34 Å <sup>o</sup> |
| H(11)  | H(22)  | 2.36                | H(72)  | H(192) | 2.26                |
| H(11)  | H(101) | 2.25                | H(81)  | H(111) | 2.25                |
| H(11)  | H(201) | 2.32                | H(81)  | H(192) | 2.32                |
| H(11)  | H(202) | 2.10                | H(81)  | H(193) | 2.29                |
| H(12)  | H(22)  | 2.40                | H(111) | H(141) | 2.27                |
| H(12)  | H(191) | 2.20                | H(111) | H(203) | 2.22                |
| H(21)  | H(172) | 2.25                | H(112) | H(121) | 2.35                |
| H(22)  | H(31)  | 2.38                | H(112) | H(181) | 2.07                |
| H(61)  | H(72)  | 2.37                | H(121) | H(181) | 2.32                |
| H(61)  | H(04)  | 2.38                | H(122) | H(201) | 2.18                |
| H(71)  | H(81)  | 2.28                | H(181) | H(191) | 2.32                |
| H(71)  | H(111) | 2.21                | H(193) | H(202) | 2.25                |

\* excluding H...H contacts for hydrogen atoms attached to the same carbon atom. The e.s.d. is between 0.03 and 0.04 Å for each contact.



In the case of the bonds C(1)-C(2), C(2)-C(3), C(6)-C(7) and C(11)-C(12), the majority of the gauche interactions are of the kind hydrogen...hydrogen or hydrogen...methylene. Because the Van der Waals radius of hydrogen is approximately one-half that of methylene, these interactions should not contribute to bond lengthening.

The presence of two adjacent axial alkyl substituents on each side of the B ring (including C(1) and C(18)) plus the equatorial C(20) methyl substituent results in a large number of close non-bonding intramolecular hydrogen-hydrogen contacts less than  $2.4 \text{ \AA}$ . These contacts are listed in Table 2.9. The large size of the C(9)-C(11)-C(12) angle ( $117.8^\circ$ ) may be the result of crowding of H(181) with H(112) and H(121). Crowding of the C(1) methylene substituent with the C(8) and C(9) methyl substituents could account for the large C(9)-C(8)-C(19) angle of  $116.8^\circ$ .

An examination of the torsion angles about ring bonds plus the valence angles subtended by successive ring bonds indicates that both A and B rings are flattened. (See Table 2.8, Figure 2.6 and Figure 2.7). This distortion increases the separation between adjacent axial substituents, thereby giving them a more equatorial disposition. The average values for the covalent bond angles in the A and B rings are  $112.1$  and  $112.4^\circ$  respectively.

The bond distances and angles observed in the lactone moiety are consistent with the geometries of the butenolide moieties of ouabain, digitoxigenin (18) and  $\Delta$ -8,14-anhydrodigitoxigenin (19) which were compared in Table 1.9 of the previous chapter. The C(15)-O(15) distance of  $1.212(4) \text{ \AA}$  is typical of carbonyl bond



distances in aldehydes and ketones in which the carbonyl oxygen does not participate as a hydrogen bond acceptor. The C(15)-O(16) distance of 1.336(4) Å is consistent with the observed shortening of the carbon-oxygen single bond involving the carbonyl carbon atom of ester moieties (50). In addition, the C(16)-O(16) distance of 1.452(4) Å is longer than the C(sp<sup>3</sup>)-O distance of 1.426 Å. The overall character of this butenolide group is therefore one of a normal ester.

The C(18)-O(17) and C(17)-O(17) distances of 1.409(3) and 1.437(4) Å respectively, are significantly different from each other. This phenomenon is characteristic of pyranose and furanose structures (53) for which one or both of the anomeric carbon-oxygen bond distances are significantly shorter than the remaining carbon-ring oxygen bond distance. The magnitude of the disproportionation tends to vary depending whether the structure corresponds to a free sugar or a glycoside and whether the C(1) non-hydrogen substituent is axial ( $\alpha$ ) or equatorial ( $\beta$ ). The magnitude of the disproportionation of the carbon-ring oxygen bond distances in the acetal ring (0.018 Å) is approximately 25% of that observed in the gutierolide butenolide ring (0.116 Å). Although the variation of the C(18)-O(17), C(18)-O(18) and C(17)-O(17) distances is in qualitative agreement with similar bonds in carbohydrates, a quantitative comparison is not justified due to the steric constraints imposed on the acetal ring by the A/C ring fusion. In contrast to the carbon-oxygen single bond distances already mentioned, the C(4)-O(4) and C(21)-O(18) bond lengths of 1.426 and 1.431(4) Å respectively, are normal.

The bond angles observed in the gutierolide ring are similar



to the corresponding angles observed in the furanose moieties of adenosine (80) and 3'-O-acetyladenosine (81) which are listed in Table 2.10.

Table 2.10

A comparison of the bond angles in the gutierolide acetal ring and the furanose rings of adenosine and 3'-O-acetyladenosine.

| Angle             | Gutierolide           | Adenosine             | 3'-O-acetyl-adenosine |
|-------------------|-----------------------|-----------------------|-----------------------|
| O(17)-C(18)-C(5)  | 104.2(2) <sup>°</sup> | 107.3(3) <sup>°</sup> | 105.3(3) <sup>°</sup> |
| C(18)-C(5) -C(4)  | 97.2                  | 101.4                 | 101.2                 |
| C(5) -C(4) -C(17) | 102.7                 | 102.7                 | 103.7                 |
| C(4) -C(17)-O(17) | 106.7                 | 104.7                 | 106.7                 |
| C(17)-O(17)-C(18) | 108.0                 | 110.6                 | 108.9                 |

Covalent bond distances involving hydrogen atoms are listed in Table 2.11. The weighted mean carbon-hydrogen bond distance for 30 C-H bonds is 0.98 Å. This value is shorter than the inter-nuclear separation of 1.10 Å for the carbon-hydrogen covalent bond (6) but is typical of structures which have been determined by X-ray diffraction methods. Bond angles of the type C-C-H and H-C-H are within 8° of the expected value (109.5°) with the exception of the angle H(21)-C(2)-H(22) which is 123°. The hydrogen atoms of the three methyl substituents are ordered. The conformation of each of the methyl groups is such that the carbon-hydrogen bonds are staggered with respect to the covalent bonds to the adjacent





Table 2.11

Covalent Hydrogen Bond Distances  
in Gutierolide.

| Bond          | Distance ( $\overset{\circ}{\text{\AA}}$ ) | Bond          | Distance ( $\overset{\circ}{\text{\AA}}$ ) |
|---------------|--|---------------|--|
| C(1) -H(11)   | 0.93(3)                                    | C(14) -H(141) | 0.96(3)                                    |
| C(1) -H(12)   | 0.93(2)                                    | C(16) -H(161) | 1.10(3)                                    |
| C(2) -H(21)   | 0.94(3)                                    | C(16) -H(162) | 0.91(3)                                    |
| C(2) -H(22)   | 1.04(3)                                    | C(17) -H(171) | 0.95(3)                                    |
| C(3) -H(31)   | 0.94(2)                                    | C(17) -H(172) | 1.01(3)                                    |
| C(6) -H(61)   | 0.94(3)                                    | C(18) -H(181) | 1.00(2)                                    |
| C(6) -H(62)   | 0.97(2)                                    | C(19) -H(191) | 0.99(3)                                    |
| C(7) -H(71)   | 0.98(3)                                    | C(19) -H(192) | 1.05(3)                                    |
| C(7) -H(72)   | 0.99(3)                                    | C(19) -H(193) | 0.97(3)                                    |
| C(8) -H(81)   | 1.02(3)                                    | C(20) -H(201) | 0.99(3)                                    |
| C(10) -H(101) | 0.95(3)                                    | C(20) -H(202) | 0.98(3)                                    |
| C(11) -H(111) | 1.00(2)                                    | C(20) -H(203) | 1.02(3)                                    |
| C(11) -H(112) | 0.95(3)                                    | C(21) -H(211) | 1.11(3)                                    |
| C(12) -H(121) | 0.98(3)                                    | C(21) -H(212) | 0.88(3)                                    |
| C(12) -H(122) | 1.02(3)                                    | C(21) -H(213) | 0.96(3)                                    |
| O(4) -H(O4)   | 0.93(3)                                    |               |  |



bonded atom.

The bond angles and interatomic distances pertaining to the intramolecular hydrogen bond  $\text{O}(4)\cdots\text{H}(\text{O}4)\cdots\text{O}(18)$  are depicted in Figure 2.8. The  $\text{O}(4)\text{--H}(\text{O}4)$  bond distance of  $0.93(3) \text{ \AA}$  falls within the range  $0.87$  to  $1.06 \text{ \AA}$  listed by Donohue (58) for hydrogen bonded systems of the type  $\text{O--H}\cdots\text{O}$ .

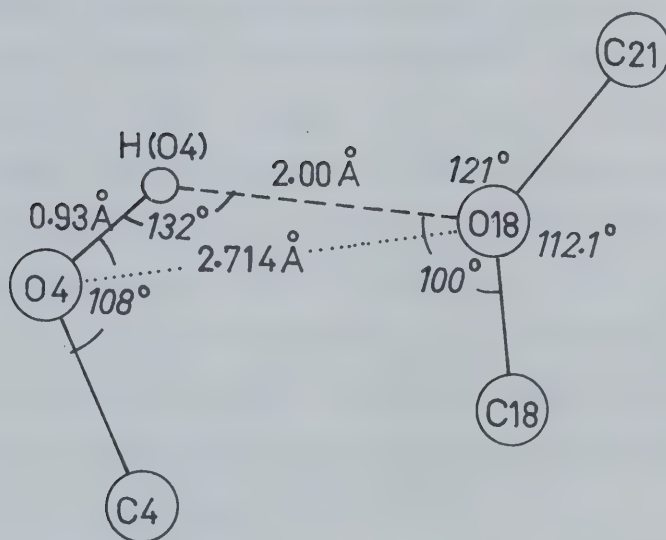


Figure 2.8 The intramolecular hydrogen bond.

Although the  $\text{O}(4)\cdots\text{O}(18)$  separation of  $2.714(3) \text{ \AA}$  falls within the range of oxygen-oxygen distances ( $2.52$  to  $2.88 \text{ \AA}$ ) listed for hydrogen bonds of this kind, the  $\text{O--H}\cdots\text{O}$  angle is  $132^\circ$ . This value is near the lower end of the distribution of  $\text{O--H}\cdots\text{O}$  angles



tabulated by Hamilton (59) which have a mean value of  $165^\circ$ . Formation of a linear hydrogen bond between O(4) and O(18) is not favored as this would require a C(4)-O(4)-H(04) angle of  $78.7^\circ$ . Although the H(04)···O(18)-C(21) and H(04)···O(18)-C(18) angles are  $121^\circ$  and  $100^\circ$  respectively, neither value is incompatible with the ability of O(18) to act as a hydrogen bond acceptor. Donohue (58) has found that the O-H vector is not necessarily directed towards an unshared electron pair on the oxygen atom assuming the atomic orbitals of oxygen to have a tetrahedral disposition. Donohue also states that hydrogen bonds of the type O-H···O are usually bent and that the maximum allowable H-O···O angle is near  $30^\circ$ . In gutierolide this intramolecular hydrogen bond also tends to obey this empirical observation as the H(04)-O(4)···O(18) angle is  $32(2)^\circ$ .

Although the Cl(3)···O(4) separation of  $3.012 \text{ \AA}$  is less than  $3.2 \text{ \AA}$ , the sum of the Van der Waals radii for chlorine and oxygen (45), no evidence was found in the final difference Fourier to suggest the existence of an intramolecular hydrogen bond with chlorine as the hydrogen bond acceptor.

In order to test the hypothesis of planarity for selected atoms within each of the ring moieties, a number of least squares planes were calculated which are described in Table 2.12.

Although the carbon atoms for all sets of alternating ring bonds in an undistorted chair conformation cyclohexane ring define a plane, none of the corresponding four atom sets in either six-membered ring satisfy this hypothesis. Both rings show a twisted distortion which is maximal for the bonded atom pairs C(1)-C(2), C(4)-C(5) in the A ring and C(6)-C(7), C(9)-C(10) in the B ring.



Table 2.12

Gutierrolide least-squares planes. Each plane has the general form  $ax + by + cz = P$  where the coefficients  $a$ ,  $b$  and  $c$  are the direction cosines of the plane with respect to the  $x$ ,  $y$  and  $z$  axes of the unit cell.

| Planar coefficients X 10 <sup>4</sup> |      |       |       | Exoplanar deviations (Å X 10 <sup>3</sup> ) |      |       |      |       |      |       |       |         |        | χ <sup>2</sup> |
|---------------------------------------|------|-------|-------|---|------|-------|------|-------|------|-------|-------|---------|--------|----------------|
| Ring                                  | A    | b     | c     | P   | C(1) | C(2)  | C(3) | C(4)  | C(5) | C(6)  | C(10) |         |        |                |
| Ring A                                | i    | -5766 | 7438  | 29653                                       | 65   | -69   | 614* | 52    | -48  | -606* |       | 1833.9  |        |                |
|                                       | ii   | -8709 | 4876  | -39898                                      | 668* | -47   | 40   | -558* | -33  | 35    |       | 853.3   |        |                |
|                                       | iii  | -7803 | 3497  | -64717                                      | 21   | -695* | -19  | 17    | 561* | -16   |       | 168.1   |        |                |
| Ring B                                | iv   | -1057 | -7965 | 117757                                      |      |       |      |       |      |       |       |         | 480.3  |                |
|                                       | v    | 5456  | -7133 | -4398                                       | 27   | -33   | 663* | 31    | -28  | -559* |       | 1552.1  |        |                |
|                                       | vi   | 2490  | -9542 | -1657                                       | 621* | -56   | 60   | -604* | -48  | 48    |       | 350.4   |        |                |
|                                       |      |       |       |   | -22  | -670* | 28   | -25   | 557* | 22    |       |         |        |                |
| Ring C                                | vii  | 516   | 413   | -9978                                       |      |       |      |       |      |       |       |         | 2483.4 |                |
|                                       | viii | 654   | 2993  | -9519                                       | 86   | -52   | -114 | 32    | 615* |       |       | 15726.8 |        |                |
|                                       | ix   | 1744  | 1789  | -9683                                       | 220  | -201  | -181 | -401* | 143  |       |       | 24846.7 |        |                |
|                                       | x    | 3929  | 1392  | -9090                                       | 163  | -263  | -2*  | -100  | 315  |       |       | 2096.1  |        |                |
|                                       | xi   | 1815  | 3301  | -9263                                       | -50  | -692* | 103  | -46   | 56   |       |       | 16402.0 |        |                |
|                                       | xii  | 2407  | 858   | -9668                                       | 462* | -128  | 182  | -134  | 250  |       |       | 0.0     |        |                |
|                                       |      |       |       | 26300                                       | 0    | -408* | 0    | 0     | 328* |       |       |         |        |                |
| Ring D                                | xiii | 2212  | -225  | -9750                                       |      |       |      |       |      |       |       |         | 2.2    |                |
|                                       | xiv  | 2178  | -258  | -9756                                       | -87* | -3    | 3    | -17*  | -2   | 3     |       | 2       | 12.8   |                |
|                                       | xv   | 2250  | -31   | -9754                                       | -95* | -5    | 7    | -5    | 5    | -1    |       | 20      | 233.4  |                |
|                                       | xvi  | 2132  | -9    | -9770                                       | -27  | 27    | 14   | -54*  | -16  | 20    |       | -11     | 363.2  |                |
|                                       |      |       |       |   | -37  | 28    | 32   | -19   | 5    | 11    |       | -6      |        |                |
|                                       |      |       |       |   |      |       |      |       |      |       |       |         |        |                |

\* atom not included in the calculation of the plane





The acetal ring exhibits a twist conformation which can be described as C(5)-exo, C(18)-endo where 'endo' indicates C(18) to be on the same side of the plane defined by C(4), C(17) and O(17) as the atoms O(4) and O(18).

The butenolide ring atoms are all within  $0.003 \text{ \AA}$  of the plane defined by equation xiii. The carbonyl oxygen atom does not deviate significantly from this plane and the dihedral angle between the planes xiii and xiv is  $0.3^\circ$ . In each of these planes C(12) is significantly exoplanar.

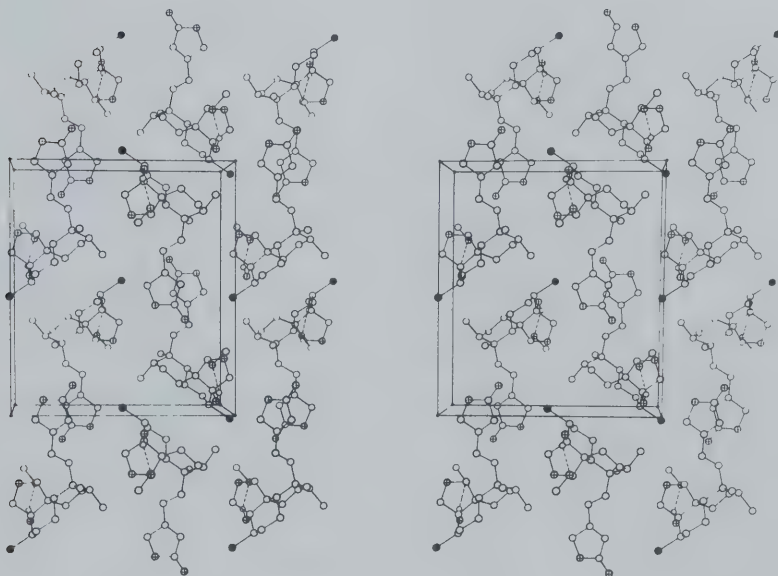


Figure 2.9 The arrangement of the guttierolide molecules in the unit cell as viewed along the z-axis. The origin of the unit cell is the upper left hand corner of the enclosure. The y-axis is down the page, the x-axis points to the right.



The crystal packing as viewed down the z-axis is shown in the stereo diagram of Figure 2.9. The arrangement of the molecule in the unit cell excludes any possibilities of intermolecular hydrogen bond formation. The butenolide ring is very nearly perpendicular to the z-axis and is located very close to the screw axis at  $1/4, 0, z$ . The butenolide rings are stacked along this screw axis with a separation between  $3.7$  and  $3.9 \text{ \AA}$ . This contact is approximately twice the Van der Waals half thickness of  $1.70 \text{ \AA}$  (44) for an aromatic ring.



## CHAPTER 3

THE CRYSTAL AND MOLECULAR STRUCTURE OF  
THREE SUBSTITUTED BENZYLETHYLMETHYLSULFONIUM PERCHLORATES3.1 INTRODUCTION

Compounds having the formula  $R_3S^+ X^-$  where R denotes an aryl or alkyl substituent joined by a single covalent bond to a sulfur atom having a unit positive charge are known as sulfonium salts. The geometry of the sulfonium cation is pyramidal with the sulfur atom at the apex of the pyramid and the  $\alpha$ -carbon atoms of the three substituents located at the corners of the trigonal base.

Sulfonium salts containing three different substituents such as  $CH_3$ ,  $C_2H_5$  and  $C_6H_5$  possess optical activity as the three substituents may be arranged in a chiral manner. Unlike amines for which a rapid dynamic equilibrium between the two pyramidal enantiomorphs prevents a chemical resolution of optically-active enantiomers, optically active sulfonium salts occur naturally in biological systems (82) and can be obtained by a chemical resolution of diastereoisomers. The first isolation of an optically active sulfonium salt was accomplished in 1900 by Pope and Peachey (83) who resolved the d- and l-methylethylcarboxymethyl-sulfonium cations as the d-camphorsulfonate salts.

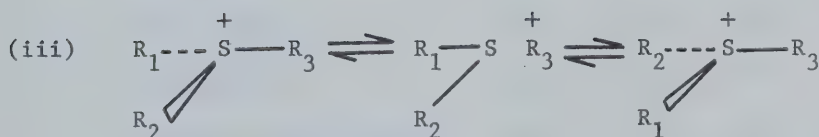
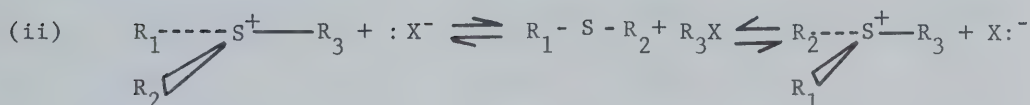
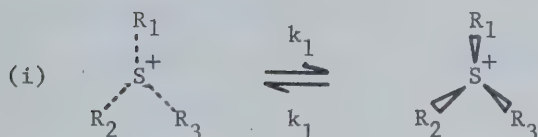
Racemization of optically active sulfonium ions in solution is known to occur by three mechanisms (84).

- (i) Pyramidal inversion about the sulfur atom without the scission of a carbon-sulfur bond.
- (ii) Reversible nucleophilic displacement at carbon of a sulfide  $R_1SR_2$ .

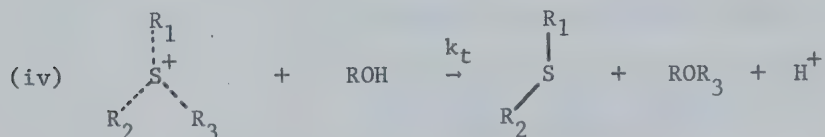


(iii) Heterolysis of a carbon sulfur bond to form a carbonium ion-neutral molecule pair which collapses to form a racemate.

Mechanisms (i), (ii) and (iii) are illustrated below.



In each case the racemization reaction may be accompanied by a solvolytic decomposition of the cation according to equation (iv)



A comparison of the activation parameters and rate constants for the pyramidal inversion of a number of aralkylethylmethylsulfonium perchlorate salts is given in Table 3.1.1 (84-86). Whereas I to X are





Table 3.1.1

Activation parameters and rate constants for the  
pyramidal inversion of  $\text{R}-\overset{\text{CH}_3}{\underset{\text{CH}_3}{\text{C}}}-\text{C}_2\text{H}_5\text{CO}_2^-$  at 50 C.

|      | R   | Solvent                          | $10^6 k_1$<br>(Sec <sup>-1</sup> ) | $\Delta H^\ddagger$ (kcal/<br>mole) | $\Delta S^\ddagger$ reference<br>(e.u.) |      |
|------|---|----------------------------------|------------------------------------|-------------------------------------|---|------|
| I    | t-butyl   | C <sub>2</sub> H <sub>5</sub> OH | 208                                | 25.0                                | 2                                       | (84) |
| II   | t-butyl   | H <sub>2</sub> O                 | 133                                | 25.9                                | 4                                       | (84) |
| III  | t-amyl  | C <sub>2</sub> H <sub>5</sub> OH | 750                                | 24.5                                | 3                                       | (84) |
| IV   | -C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>                      | C <sub>2</sub> H <sub>5</sub> OH | 401                                | 25.4                                | 4                                       | (84) |
| V    | 1-adamantyl   | CH <sub>3</sub> COOH             | 430                                | 26                                  | 7                                       | (84) |
| VI   | C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>   | CH <sub>3</sub> OH               | 3.48                               | 27.6                                | 2                                       | (84) |
| VII  | p-NO <sub>2</sub> C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                         | CH <sub>3</sub> OH               | 3.34                               | 28.1                                | 3                                       | (84) |
| VIII | p-ClC <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                                       | CH <sub>3</sub> OH               | 3.23                               | 29                                  | 7 ± 1                                   | (85) |
| IX   | m-NO <sub>2</sub> C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                         | CH <sub>3</sub> OH               | 3.25                               | 28.6                                | 6                                       | (85) |
| X    | p-CH <sub>3</sub> C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                         | CH <sub>3</sub> OH               | 3.38                               | 29.5                                | 9 ± 2                                   | (85) |
| XI   | 3,5-diCH <sub>3</sub> -4-OCH <sub>3</sub> C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> | CH <sub>3</sub> OH               | 6.1*                               | 25.3                                | -3 ± 6                                  | (85) |
| XII  | 4-OCH <sub>3</sub> C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                        | CH <sub>3</sub> OH               | 154**                              | -                                   | -                                       | (86) |

\* mechanism not definitely known

\*\* mechanism of inversion suggested  
to be (iii), rate constant is for  
first order racemization.



believed to racemize predominantly in polar solvents at 50°C by mechanism (i), the large value of  $k_1$  listed for XII does not agree with the values of  $k_1$  for the analogous compounds VI to X and is one to two orders of magnitude larger. This suggests that the major pathway for its racemization is not mechanism (i) and Darwish, Hui and Tomlinson (86) have postulated racemization mainly by mechanism (iii). Racemization of XI is probably via inversion at the sulfur pole but Po states that other pathways may be present (85).

Sulfonium salts having three primary alkyl substituents invert at a slower rate than sulfonium salts containing a bulky *t*-butyl, *t*-amyl or 1-adamantyl substituent. Although Anderson and Papanikolaou (84) were unable to prepare optically active phenyl-*o*-anisyl-*p*-tolyl-sulfonium perchlorate, Anderson (84) has prepared 9,9-dimethyl-10-phenylthioxanthylum perchlorate and observed from NMR spectra that the two methyl group resonances coalesce at  $200 \pm 5^\circ$  in benzophenone. The activation energy for the equilibration of the two methyl substituents was estimated to be 25.4 kcal/mole. All of this evidence suggests that the rate of inversion is highly susceptible to steric effects and that increased non-bonded interactions among the three substituents will result in a lower barrier to inversion with the geometry of the ground state tending to approach that of the transition state. Although the geometry of the latter is not necessarily planar, somewhere along the reaction coordinate and very close to the transition state, the three carbon-sulfur bonds must lie in a plane.

Kincaid and Hendriques (87) calculated inversion barriers for several pyramidal species of the kind  $XY_3$  in order to predict whether or not similar compounds with three non-identical Y substituents could



possess optical activity at room temperature. The parabolic function  $V = kx^2/2$  was used.  $V$  is the barrier to inversion in kcal/mole;  $x$  is the height of the pyramid in cm;  $k$  is the force constant for the symmetrical deformation vibrations which lead to inversion.  $K$  is defined by  $k = 4\pi^2 \nu^2 \mu$  where  $\mu$  is the reduced mass of the system,  $(3M_X M_Y / (3M_Y + M_X))$  and  $\nu$  is the symmetric deformation vibrational frequency in  $\text{cm}^{-1}$ .

Estimates of  $V$  obtained in this manner in kcal/mole were: ammonia 11, phosphine 47, trimethylamine 15, and trimethylsulfonium, 100. The values of  $\nu$  for  $(\text{CH}_3)_3\text{S}^+$  was  $600 \text{ cm}^{-1}$ , the C-S bond distance was  $1.82 \text{ \AA}$  and the C-S-C angles were  $109.5^\circ$  each. Even if the inversion barrier is in error by a factor of 2 this estimate suggests that the trimethylsulfonium cation will not invert at room temperature.

Lower values of  $V$  have been obtained for  $(\text{CH}_3)_3\text{S}^+$  and other pyramidal species of the type  $\text{XY}_3$  using the valency force field equation of Costain and Sutherland (88).

$$V = 3/2 [k_1(\Delta\ell)^2 + k_\delta(\Delta\alpha)^2]$$

where  $k_1$  and  $k_\delta$  are the force constants obtainable from  $\nu_1$  and  $\nu_2$ , the symmetrical stretching and bending frequencies respectively;  $(\Delta\alpha)$  and  $(\Delta\ell)$  are the changes in the Y-X-Y angle and X-Y bond length in going from the equilibrium ground state to the planar transition state. This expression obtained satisfactory values of  $V$  for ammonia ( $V = 6.0 \text{ kcal/mole}$ ) which were in agreement with spectroscopic values (88).

Koepl, Sagatys, Krishnamurthy and Miller (89) have compiled a rather extensive list of inversion barriers for pyramidal ions and molecules of the kind  $\text{XY}_3^\pm$  using the method of Costain and Sutherland. Inversion barriers estimated in this manner include  $\text{NH}_3$ , 5.58;  $\text{PH}_3$ , 27;



$\text{N}(\text{CH}_3)_3$ , 7.46;  $\text{P}(\text{CH}_3)_3$ , 20.4;  $\text{AsH}_3$ , 34 and  $(\text{CH}_3)_3\text{O}^+$ , 19.2 kcal/mole.

V was also calculated as a function of  $\nu_1$ ,  $\nu_2$ ,  $\ell$ ,  $\alpha$ ,  $M_X$  and  $M_Y$  keeping five variables fixed at the literature value and allowing only one parameter to vary. Their results indicated that estimates of V are insensitive to changes in  $M_X$ ,  $\nu_1$  and  $\ell$  but increase with  $\nu_2$  and decrease with increasing values of  $\Delta\alpha$  and  $M_Y$ . Several values which were obtained for inversion of the trimethylsulfonium cation are listed in Table 3.1.2.

Table 3.1.2

Estimates of the Inversion Barrier for  $(\text{CH}_3)_3\text{S}^+$  (89).

| $\begin{array}{c} \text{C} \quad \text{O} \quad \text{S} \\ \diagup \quad \diagdown \\ \text{A} \end{array}$ | $\alpha$<br>degrees | $\nu_1$<br>$\text{cm}^{-1}$ | $\nu_2$<br>$\text{cm}^{-1}$ | V<br>kcal/mole |
|--|---------------------|-----------------------------|-----------------------------|----------------|
| 1.820  | 109.08              | 654                         | 285                         | 9.2            |
| 1.830  | 103.0               | 654                         | 285                         | 18.            |
| 1.820  | 100.0               | 654                         | 285                         | 23             |
| 1.830  | 100.08              | 654                         | 285                         | 23             |

Koepl et al (89) suggest that neutral  $\text{XY}_3$  species having values of V less than 22 kcal/mole will not have resolvable optical isomers whereas elements of the second period and higher periods should be capable of optical activity. Although Darwish (84) suggests that a more precise description of the geometry of the sulfonium pole is necessary to obtain better estimates of V, it has been pointed out by Koepl et al that estimates obtained in this manner will not account for the extra stabilization of the pyramidal ground state due to interactions of the polar solvent with the charged species. For this reason the sulfonium





cation will invert less readily than indicated from the above model.

Several crystallographic structure determinations (90-92) have shown carbon-sulfur bond distances in sulfonium salts ranging from 1.80 to  $1.84 \text{ \AA}$  and C-S-C angles between  $100$  and  $105^\circ$ . Lopez-Castro and Truter (90) suggest the sulfur atom in dimethylphenylsulfonium perchlorate is  $sp^3$  hybridized with three of the hybrid orbitals forming  $\sigma$ -bonds with carbon and the lone pair of electrons occupying the fourth orbital. The discrepancy between the observed C-S-C angles ( $102$ ,  $103$  and  $105(1)^\circ$ ) and the tetrahedral value of  $109.5^\circ$  was attributed to atom-lone pair repulsions being greater than atom-atom repulsions. Price and Oae (93) suggest that in sulfonium salts, the 3p orbitals of sulfur are used in  $\sigma$ -bond formation with the lone electron-pair occupying the 3s orbital of sulfur. Steric repulsions between substituents would account for angles larger than  $90^\circ$ . In 1-acetonyl-1-thionia-5-thiocyclooctane (91), the sulfonium pole was thought to have a trigonal bipyramidal geometry due to the presence of two short sulfur-carbonyl oxygen and sulfur-sulfur contacts of  $2.81 \text{ \AA}$  and  $3.12 \text{ \AA}$  respectively.

In order to relate the structural properties of the sulfonium salts to the kinetic data for their pyramidal inversion (84-86), the crystal structures of the following three sulfonium salts are presented in sections 3.2, 3.3 and 3.4 of this chapter.

(d,  $\ell$ )-4-nitrobenzylethylmethylsulfonium perchlorate

( $\ell$ )-3,5-dimethyl-4-methoxybenzylethylmethylsulfonium perchlorate

(d)-4-chlorobenzylethylmethylsulfonium perchlorate



The choice of anion was restricted to  $\text{ClO}_4^-$  or (-) dibenzoyltartrate as the chloride, bromide and methylsulfate salts were difficult to crystallize, tending to form oils. The dibenzoyltartrates were not chosen due to the predominant wafer habit of these crystals and the large number of atoms in comparison to the perchlorate. Two optically active salts were chosen because the presence of the anomalous scattering powers of the elements sulfur and chlorine would allow the determination of the chirality of the sulfonium pole. Unlike the related pyrimidal sulfoxides for which a number of absolute configuration studies have been performed by crystallographic or stereospecific synthetic methods (94,95), no data are available on the absolute configuration of sulfonium salts.

### 3.2 THE CRYSTAL STRUCTURE OF (d,l)-4-NITROBENZYLETHYLMETHYLSULFONIUM PERCHLORATE

#### 3.2.1 Experimental

Crystals of racemic 4-nitrobenzylethylmethylsulfonium perchlorate were obtained in the form of pale yellow needles from Dr. D. Darwish. Vapor diffusion of diethyl ether into a cold methanolic solution of the sulfonium salt yielded rhombic prisms exhibiting 2/m morphology and showing the prominent crystal forms  $\{0\ 0\ 1\}$  and  $\{1\ 1\ 0\}$ .

Preliminary diffraction photographs showed 2/m diffraction symmetry and the following systematically unobserved reflections -  $h0\ell$ , absent for  $\ell = 2n + 1$ ;  $0k0$ , absent for  $k = 2n + 1$ ;  $hkl$ , none. Unit cell dimensions were determined from the angular coordinates of 12 reflections by the least-squares method of Busing and Levy (33). Crystallographic data and experimental details of the data collection are listed in Table 3.2.1.



Table 3.2.1

Crystallographic data and experimental details of the data collection.

(a) Physical constants and unit cell data.

|                        |   |
|------------------------|---|
| Formula                | $C_{10}H_{14}O_6NSCl$   |
| Molecular weight       | 311.8   |
| Space group            | $P2_1/c$  |
| Cell dimensions        | $a = 12.066(1) \text{ \AA}$<br>$b = 9.031(1)$<br>$c = 13.668(1)$<br>$\beta = 111.05(2)$ |
| Volume                 | $V = 1390.0 \text{ \AA}^3$  |
| Density                | observed $1.48(1) \text{ g/cm}^3$<br>calculated $1.489 \text{ g/cm}^3$                  |
| Cell content           | $z = 4$ molecules/unit cell   |
| Absorption coefficient | $\mu = 39.9 \text{ cm}^{-1} \text{ (Cu K}\alpha\text{)}$                                |

(b) Experimental details of the data collection.

|                                    |   |
|------------------------------------|---|
| Radiation                          | Ni-filtered Cu K  |
| Specimen size                      | $0.22 \times 0.20 \times 0.30 \text{ mm (a} \times \text{b} \times \text{c)}$ |
| Scan mode                          | $\theta/2\theta$ moving crystal/moving detector                               |
| Scan rate                          | $2^\circ 2\theta/\text{min.}$   |
| Range of reciprocal space explored | 1 quadrant $(hkl, \bar{h}k\ell)$ with<br>$3^\circ < 2\theta < 129.0^\circ$    |
| No. of reflections measured        | 2356  |
| No. of reflections observed        | 1897  |
| Final conventional residual R      | 0.062   |
| Final weighted residual $R_w$      | 0.090   |



The intensity data were measured using Ni-filtered Cu K $\alpha$  radiation by a technique similar to that described in the previous chapter. During the data collection the average decrease in the intensities of the three standard reflections was 2.8% of the initial values. The crystal was immersed in liquid nitrogen prior to being used in the data collection.

The intensity data were corrected for decomposition, Lorentz and polarization effects and 1897 reflections with  $I > 3\sigma(I)$  were considered observed. The data were corrected for absorption using the program NRC-3 (35) which employs the m-point Gaussian integration formula of Busing and Levy (96). The crystal was divided into seven subdivisions in each direction. The evaluation of the transmission factor required approximately 0.36 seconds of C.P.U. time on the IBM 360/67 computer for each reflection.

Prior to the acquisition of the Picker FACS-1 diffractometer a set of structure factors was derived from photographic data obtained from Weissenberg films and measured visually by comparison with a linear intensity scale. This data was used only in the initial stage of the structure determination as it was known to contain serious errors due to crystal decomposition.

The Wilson plot (36) estimates for the scale and overall temperature factor for the photographic data were 1.76 and  $B=3.21 \text{ \AA}^2$ . A sharpened Patterson synthesis was computed with the coefficients  $|F|^2$  modified to correspond to point atoms at rest (97).

The determination of the sulfur and chlorine atomic coordinates was facilitated by the following relationships for the vectors between symmetry related atoms.





| Vector           | Coordinate |                     |                    | Relative peak height |
|------------------|------------|---------------------|--------------------|----------------------|
| Harker line      | 0,         | $\frac{1}{2} + 2y,$ | $\frac{1}{2}$      | 2                    |
| Harker section   | 2x,        | $\frac{1}{2},$      | $\frac{1}{2} + 2z$ | 2                    |
| General position | 2x,        | 2y,                 | 2z                 | 1                    |

Two sets of self-consistent prominent vectors were tentatively identified as the chlorine-chlorine and sulfur-sulfur vectors. The set containing the largest peaks was used to determine the coordinates of the chlorine atom. The ambiguity in the position of the sulfur atom was resolved by correctly identifying all sulfur-chlorine cross vectors. The coordinates of the two atoms were

|    | x/a   | y/b    | z/c   |
|----|-------|--------|-------|
| S  | 0.174 | -0.028 | 0.340 |
| Cl | 0.196 | 0.320  | 0.149 |

The initial value of the residual for the structure factor calculation using these coordinates was 0.49. The remaining 17 non-hydrogen atoms were readily located by heavy-atom Fourier techniques. The value of the residual for the structure factor calculation using the diffractometer data uncorrected for absorption and the unrefined parameters of 19 atoms was 0.32.

The parameter refinement was performed by the method of full matrix least-squares using a locally modified version of ORFLS (98) until the final eight cycles of least-squares which were performed with NRC-10 (35). The function minimized was

$$D = \sum w ( |F_o| - |F_c| )^2.$$



The positional and isotropic thermal parameters of all 19 atoms were refined for three cycles of least-squares with  $w = 1.0$  for each reflection. The thermal parameters were converted to an anisotropic model at the residual 0.141 and refined as such for an additional three cycles of least-squares. The locations of the hydrogen atoms were determined from a difference Fourier at the residual 0.082. The hydrogen atoms were assigned isotropic thermal parameters and were included in the refinement. Three additional cycles of least-squares decreased the residual from 0.071 to 0.069 and the absorption-corrected data was then introduced.

The reflections  $(2\ 0\ 0)$ ,  $(3\ 0\ 0)$ ,  $(\bar{1}\ 2\ 2)$  and  $(\bar{3}\ 1\ 2)$  showed large individual errors of the type  $|F_c| > |F_o|$ . These errors were attributed to a reduction in the value of  $|F_o|$  due to counter paralysis and/or secondary extinction. Each reflection was given a modified weight  $w'$  such that  $w'(|F_o| - |F_c|)^2 = 0.5$ . Corrections were made to the sulfur and chlorine form factors to account for the imaginary component of anomalous dispersion. Eight additional cycles of least-squares resulted in a convergence of all parameters with all calculated shifts less than 0.2 of the corresponding parameter e.s.d's. The final values of the conventional and weighted residuals were  $R = 0.062$  and  $R_w = 0.090$ .

A difference Fourier based on the final set of parameters showed several extremes ranging from 0.42 to  $-0.29\ \text{e}/\text{\AA}^3$  in the vicinity of the perchlorate ion. The difference electron density for the remainder of the unit cell was within the limits 0.17 and  $-0.17\ \text{e}/\text{\AA}^3$ . Although the perchlorate ion was somewhat disordered, the magnitude of the disordering was not severe enough to warrant further computation.



Table 3.2.2

The positional parameters ( $\times 10^4$ ) of the non-hydrogen atoms. Parameter e.s.d's are in parentheses.

| Atom  | x/a     | y/b      | z/c     |
|-------|---------|----------|---------|
| S(1)  | 1756(1) | -270(1)  | 3428(1) |
| C(1)  | 3299(5) | 163(6)   | 4036(4) |
| C(2)  | 1776(4) | -1420(5) | 2361(3) |
| C(3)  | 535(6)  | -1874(8) | 1675(4) |
| C(4)  | 1464(4) | -1552(5) | 4335(3) |
| C(5)  | 2364(3) | -2750(4) | 4781(3) |
| C(6)  | 3290(4) | -2520(5) | 5735(3) |
| C(7)  | 4086(4) | -3664(5) | 6188(3) |
| C(8)  | 3945(3) | -4988(4) | 5671(3) |
| C(9)  | 3058(4) | -5229(5) | 4709(3) |
| C(10) | 2276(4) | -4100(5) | 4276(3) |
| N(11) | 4785(3) | -6206(4) | 6131(3) |
| O(12) | 5456(4) | -6051(5) | 7046(3) |
| O(13) | 4784(3) | -7280(4) | 5608(3) |
| Cl(1) | 1955(1) | 3121(1)  | 1518(1) |
| O(1)  | 2006(7) | 3323(7)  | 528(5)  |
| O(2)  | 1175(5) | 1890(5)  | 1370(5) |
| O(3)  | 3060(4) | 2683(9)  | 2170(5) |
| O(4)  | 1474(5) | 4407(6)  | 1769(5) |



Table 3.2.3

The parameters describing the anisotropic thermal vibration of the atoms listed in Table 3.2.2. All quantities are multiplied by  $10^4$ .

| Atom  | $U_{11}^*$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{23}$ | $U_{13}$ |
|-------|------------|----------|----------|----------|----------|----------|
| S(1)  | 263(3)     | 174(2)   | 250(3)   | 72(2)    | 38(2)    | 109(2)   |
| C(1)  | 299(13)    | 221(12)  | 381(16)  | -12(10)  | 42(11)   | 126(11)  |
| C(2)  | 340(14)    | 236(12)  | 243(11)  | 86(10)   | 26(9)    | 151(10)  |
| C(3)  | 408(19)    | 404(19)  | 286(15)  | 34(15)   | -54(13)  | 85(13)   |
| C(4)  | 223(10)    | 215(10)  | 236(10)  | 40(8)    | 16(8)    | 113(8)   |
| C(5)  | 175(8)     | 181(9)   | 187(8)   | -4(7)    | 24(7)    | 78(7)    |
| C(6)  | 249(10)    | 198(10)  | 160(8)   | -15(8)   | -27(7)   | 58(7)    |
| C(7)  | 221(10)    | 224(10)  | 167(9)   | -11(8)   | 24(8)    | 34(7)    |
| C(8)  | 190(9)     | 170(9)   | 196(9)   | 1(7)     | 57(7)    | 62(7)    |
| C(9)  | 257(11)    | 150(9)   | 247(11)  | -28(8)   | 1(8)     | 59(9)    |
| C(10) | 212(9)     | 169(9)   | 200(9)   | -34(8)   | 0(7)     | 27(8)    |
| N(11) | 240(9)     | 232(10)  | 338(11)  | 4(8)     | 116(8)   | 110(8)   |
| O(12) | 455(14)    | 362(12)  | 284(10)  | 80(10)   | 98(9)    | -55(9)   |
| O(13) | 335(10)    | 214(8)   | 440(11)  | 68(7)    | 64(8)    | 162(9)   |
| Cl(1) | 210(2)     | 249(3)   | 234(2)   | 19(2)    | 45(2)    | 87(2)    |
| O(1)  | 1412(42)   | 514(20)  | 627(22)  | 162(24)  | 199(16)  | 696(26)  |
| O(2)  | 428(15)    | 328(13)  | 905(26)  | -47(11)  | 110(15)  | 231(15)  |
| O(3)  | 316(14)    | 788(30)  | 902(30)  | 27(16)   | 199(23)  | -139(17) |
| O(4)  | 563(19)    | 484(18)  | 733(22)  | 38(15)   | -252(17) | 264(16)  |

\* These coefficients are defined in the Preface.





Table 3.2.4

The positional and thermal parameters ( $\times 10^3$ ) for the hydrogen atoms of (d,l)-p-nitrobenzylethyl-methylsulfonium perchlorate.

| Atom   | x/a    | y/b     | z/c    | $U_{iso}$ |
|--------|--------|---------|--------|-----------|
| H(11)  | 350(5) | 74(7)   | 371(4) | 93(16)    |
| H(12)  | 371(4) | -67(6)  | 414(3) | 75(13)    |
| H(13)  | 336(5) | 47(7)   | 461(4) | 108(19)   |
| H(21)  | 214(4) | -74(6)  | 197(4) | 87(15)    |
| H(22)  | 225(4) | -211(5) | 262(3) | 56(10)    |
| H(31)  | 9(4)   | -109(7) | 131(4) | 97(17)    |
| H(32)  | 58(5)  | -260(7) | 114(4) | 114(21)   |
| H(33)  | 12(6)  | -255(9) | 203(6) | 135(24)   |
| H(41)  | 135(4) | -106(5) | 489(3) | 59(11)    |
| H(42)  | 62(4)  | -198(5) | 393(3) | 61(11)    |
| H(61)  | 332(4) | -157(6) | 593(3) | 77(13)    |
| H(71)  | 471(4) | -338(6) | 686(3) | 75(14)    |
| H(91)  | 301(4) | -606(5) | 425(3) | 68(12)    |
| H(101) | 161(4) | -427(6) | 373(4) | 85(15)    |





Table 3.2.5

A listing of the observed structure amplitudes and final structure factors (absolute scale X 10) for 4-nitrobenzylethylmethylsulfonium perchlorate. Unobserved reflections are designated by an asterisk (\*). Reflections which were assigned modified weights are marked with a dagger (†).











The final refined values of the atomic parameters and their calculated e.s.d's are listed in Tables 3.2.2, 3.2.3 and 3.2.4. The observed and calculated structure factors are listed in Table 3.2.5.

### 3.2.2 Results and Discussion

The conformation of the sulfonium salt, showing the orientation of the vibration ellipsoids, scaled to include 35% probability, is shown in Figure 3.2.1 which also shows the atomic numbering scheme. The perchlorate ion resembles a slightly irregular tetrahedron. The sulfonium cation exhibits pyramidal geometry with the phenyl ring in a gauche conformation with respect to the plane defined by S, C(1) and C(2). The plane defined by S, C(4) and C(5) is almost perpendicular to the phenyl ring with the C(2)-C(3) bond trans with respect to the C(1)-S bond. The nitro substituent is planar but inclined by  $11.1^\circ$  with respect to the phenyl ring.

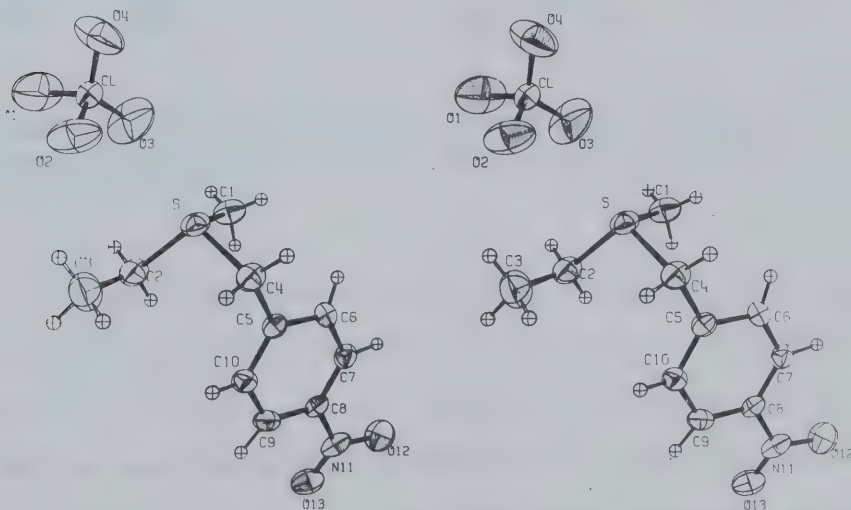


Figure 3.2.1 The molecular structure of 4-nitrobenzylethylmethylsulfonium perchlorate.



Bond distances and interbond angles excluding hydrogen atoms are depicted in Figure 3.2.2. Denoting the atoms N, C and O by X, the average e.s.d's for the angles  $SXX$ ,  $XSX$ ,  $XC\ell X$  and  $XXX$  are  $0.4$ ,  $0.3$ ,  $0.4$  and  $0.4^\circ$  respectively. Carbon-hydrogen bond distances are listed in Table 3.2.6.

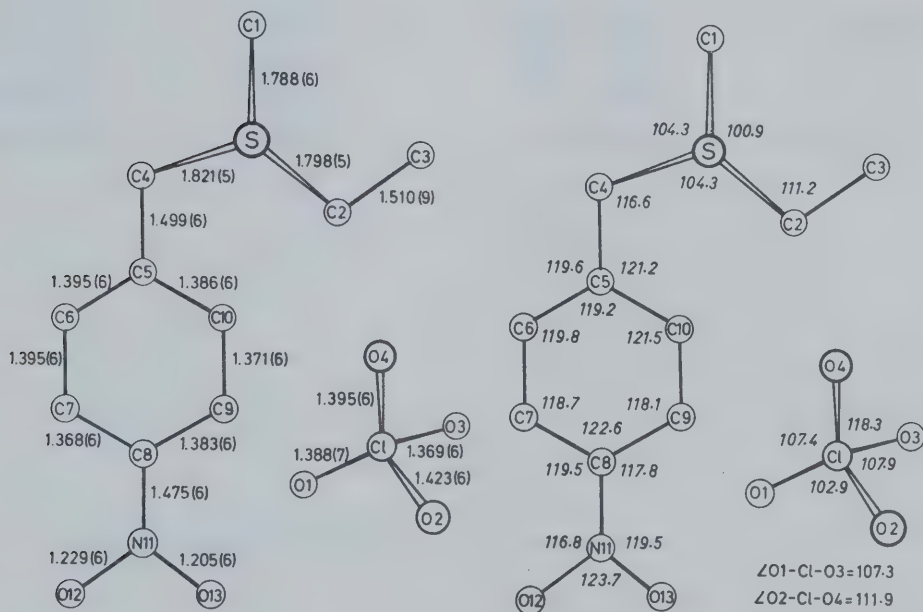


Figure 3.2.2 The bond distances and bond angles (excluding hydrogen atoms) derived from the final atomic coordinates listed in Table 3.2.2.

A number of bond distances were corrected for thermal motion according to the method of Busing and Levy (49). These corrections are listed in Table 3.2.7. The upper bound corresponds to the situation where the thermal motions of the two atoms are highly correlated and antiparallel; the lower bound corresponds to the case where the motions are highly correlated and parallel.



Table 3.2.6

## Carbon-Hydrogen Covalent Bond Distances

|            |           |              |           |
|------------|-----------|--------------|-----------|
| C(1)-H(11) | 0.78(6) Å | C(3)-H(33)   | 0.91(6) Å |
| C(1)-H(12) | 0.90(5)   | C(4)-H(41)   | 0.92(4)   |
| C(1)-H(13) | 0.81(6)   | C(4)-H(42)   | 1.05(4)   |
| C(2)-H(21) | 1.01(6)   | C(6)-H(61)   | 0.88(5)   |
| C(2)-H(22) | 0.84(5)   | C(7)-H(71)   | 0.98(5)   |
| C(3)-H(31) | 1.01(6)   | C(9)-H(91)   | 0.97(5)   |
| C(3)-H(32) | 1.02(6)   | C(10)-H(101) | 0.89(5)   |

$$\langle \text{C-H} \rangle = 0.93 \text{ Å}$$

Table 3.2.7

## Bond lengths corrected for thermal motion (49)

| Atom A | Atom B | Uncorrected<br>bond length | Lower<br>Bound | Riding<br>Motion | Upper<br>Bound |
|--------|--------|----------------------------|----------------|------------------|----------------|
| S(1)   | C(1)   | 1.788(6) Å                 | 1.790 Å        | 1.803 Å          | 1.917 Å        |
| S(1)   | C(2)   | 1.798(5)                   | 1.799          | 1.806            | 1.933          |
| C(4)   | S(1)   | 1.821(5)                   | 1.821          | 1.823            | 1.939          |
| C(2)   | C(3)   | 1.510(9)                   | 1.514          | 1.538            | 1.689          |
| Cl(1)  | O(1)   | 1.388(7)                   | 1.432          | 1.517            | 1.763          |
| Cl(1)  | O(2)   | 1.423(6)                   | 1.445          | 1.501            | 1.706          |
| Cl(1)  | O(3)   | 1.369(6)                   | 1.417          | 1.506            | 1.763          |
| Cl(1)  | O(4)   | 1.395(6)                   | 1.421          | 1.482            | 1.693          |



The value listed under the heading 'riding motion' corresponds to the case where the atomic separation is independent of the position of atom A.

Although the three carbon-sulfur bond distances of 1.821(5) Å, 1.798(5) Å and 1.788(6) Å are apparently non-equivalent, the difference is not significant for two reasons.

- (i) The e.s.d's in the bond distances and angles are probably underestimated by as much as a factor of 2.
- (ii) The methyl carbon has a large component of thermal motion perpendicular to the S-C(1) bond. Assuming the 'riding motion' approximation to be valid, the corrected S-C(1) distance is 1.803 Å. If the same correction is applied to the S-C(2) distance (resulting in the value 1.806 Å) then the average C-S single bond distance of 1.810 Å is in agreement with Sutton's tabulated value of 1.817(5) Å for a C-S single bond (6).

Including the correction of the C(2)-C(3) distance (1.510(9) Å) for 'riding motion' giving a value of 1.538 Å, all carbon-carbon bond distances agree with the literature values listed by Sutton (6). The six C(sp<sup>2</sup>)-C(sp<sup>2</sup>) distances in the phenyl ring range from 1.368 to 1.395(7) Å with a weighted mean value of 1.383 Å. The standard deviation from the mean is 0.005 Å. This average agrees with the average carbon-carbon bond length of 1.392 Å in benzene (99).

A comparison of the geometries of the nitro substituents for a number of compounds containing a p-nitrophenyl moiety has recently been published by Guttermson and Robertson (100). The bond





distances, bond angles and inclination of the plane of the nitro substituent with respect to the phenyl ring in this structure agree with those values listed in (100). In each structure, the C(7)-C(8)-C(9) angle (this structure,  $122.6^\circ$ ) is larger than the theoretical value of  $120^\circ$ . Carter, McPhail and Sim (101) have suggested that the strong electron-withdrawing power of the  $\text{NO}_2$  group results in the C-N bond having less than  $1/3s$  character. The increase in  $s$  character in the C-C bonds to the ortho ring carbons leads to an opening of the C-C-C angle.

The chlorine-oxygen bond distances are appreciably shorter than the value of  $1.46(1) \text{ \AA}$  observed in nitronium perchlorate (102), due to the large thermal motion of the oxygen atoms. Correction of these distances using the 'riding motion' approximation (49) gives an average value of  $1.501 \text{ \AA}$  (see Table 3.2.7). The discrepancies of the present results from the literature value are not unusual due to the failure of the anisotropic ellipsoid model to fully describe the oscillatory thermal motion of the perchlorate oxygen atoms. In dimethylphenylsulfonium perchlorate (90) and 1-acetyl-1-thionia-5-thiacyclooctane perchlorate (91), abnormal chlorine-oxygen distances were also observed. The O-Cl-O angles shown in Figure 3.2.1 are approximately tetrahedral with the exception of O(1)-Cl-O(2) ( $102.9^\circ$ ) and O(4)-Cl-O(3) ( $118.3^\circ$ ).

Interactions between the phenyl ring and the  $\text{C}_2\text{H}_5\text{-S-CH}_3$  moiety compress the methyl and ethyl substituents together so that the C(1)-S-C(2) angle of  $100.9^\circ$  is significantly smaller than the angles C(1)-S-C(4) and C(2)-S-C(4) which are each  $104.3(3)^\circ$ . The equivalence of these two angles is consistent with the observation that the plane



defined by S, C(4) and C(5) is roughly perpendicular to both the phenyl ring and the plane defined by S, C(1) and C(2). Similarly, Van der Waals interactions between the phenyl ring and the  $\text{CH}_3\text{-S-C}_2\text{H}_5$  substituent contribute to the large value of  $116.6(4)^\circ$  for the angle S-C(4)-C(5) which is significantly larger than the S-C(2)-C(3) angle of  $111.2^\circ$ . A number of short non-bonding intramolecular contacts which may be important in the geometry of the sulfonium pole are listed in Table 3.2.8.

Table 3.2.8

Short non-bonding contacts important  
in the geometry of the cation.

| Atom A | Atom B | Separation                       |
|--------|--------|----------------------------------|
| S(1)   | C(3)   | $2.73 \overset{\circ}{\text{Å}}$ |
| S(1)   | C(5)   | 2.83                             |
| S(1)   | C(6)   | 3.65                             |
| S(1)   | C(10)  | 3.63                             |
| C(1)   | C(6)   | 3.36                             |
| C(2)   | C(10)  | 3.46                             |
| O(12)  | H(71)  | 2.54                             |
| O(13)  | H(91)  | 2.52                             |

The Van der Waals radii (Payling (45)) for a number of species are: sulfur, 1.85 Å, oxygen, 1.4 Å; hydrogen, 1.2 Å; methyl, 2.0 Å; half-thickness of an aromatic ring, 1.70 Å. The Van der Waals radius for sulfur is for neutral S and no value is available for the Van der Waals radius of  $\text{S}^+$  which should be less than 1.85 Å.



The six atoms of the phenyl ring, N(11) and the hydrogen atoms H(61), H(71), H(91) and H(101) are all within  $0.014 \text{ \AA}$  of the plane whose direction-cosine equation is  $0.8183x + 0.3282y - 0.4718z = -3.2657$  ( $\chi^2 = 29.0$ ). The atoms C(4), O(12) and O(13) deviate by  $-0.099$ ,  $-0.212$  and  $0.203 \text{ \AA}$  from this plane. The nitro group is planar but inclined  $11.1^\circ$  with respect to the plane of the phenyl ring due to steric interactions of the two oxygen atoms with the hydrogen substituents of the ortho ring carbon atoms (see Table 3.2.8). The equation of the plane for C(8), N(11), O(12) and O(13) of the nitro group is  $0.8434x + 0.4367y - 0.3197z = -2.5641$  ( $\chi^2 = 0.001$ ). The equation of the plane defined by C(1), C(2) and C(4) is  $-0.5587x + 0.7871y - 0.2615z = -2.3474$ . The sulfur atom is  $0.767(1) \text{ \AA}$  from this plane.

The arrangement of the sulfonium cations and perchlorate anions in the unit cell is depicted in Figures 3.2.3 and 3.2.4 which are projections of the unit cell onto the  $(1\ 0\ 0)$  and  $(0\ 1\ 0)$  planes respectively. Each sulfur atom forms four  $S^+ \cdots O-ClO_3^-$  contacts less than  $3.82 \text{ \AA}$ . These contacts are shown in Figure 3.2.3. All  $S \cdots O$  distances less than  $4.5 \text{ \AA}$  are listed in Table 3.2.9.



Table 3.2.9

 $\text{S}^+ \cdots \text{O}-\text{ClO}_3^-$  distances less than  $4.5 \text{ \AA}$ 

| Atom A | Atom B  | Separation         |
|--------|---------|--------------------|
| S(1)i  | O(1)ii  | $3.29 \text{ \AA}$ |
| S(1)i  | O(2)i   | 3.29               |
| S(1)i  | O(3)i   | 3.81               |
| S(1)i  | O(3)iii | 4.46               |
| S(1)i  | O(4)iii | 3.82               |

|     |     |                     |                   |
|-----|-----|---------------------|-------------------|
| i   | x,  | y,                  | z                 |
| ii  | x,  | $\frac{1}{2} - y,$  | $\frac{1}{2} + z$ |
| iii | -x, | $-\frac{1}{2} + y,$ | $\frac{1}{2} - z$ |

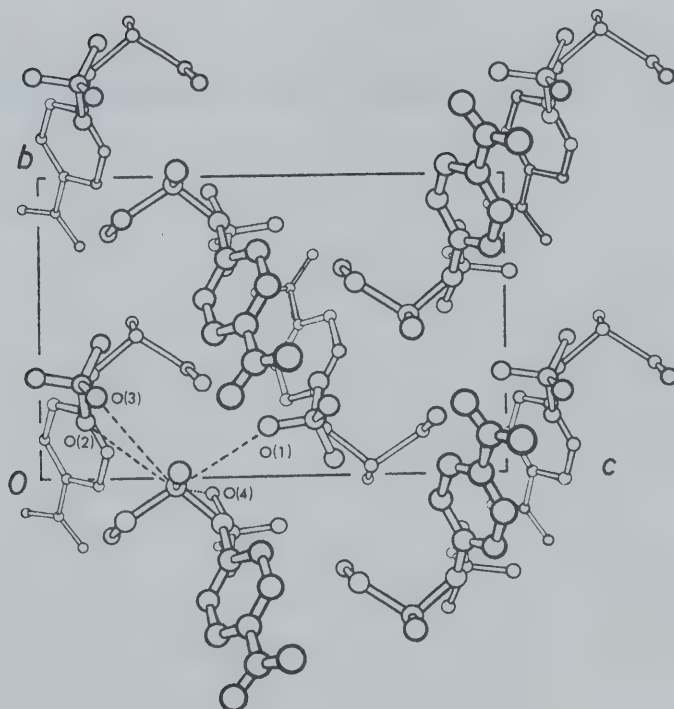


Figure 3.2.3 A projection of the unit cell contents onto (1 0 0).





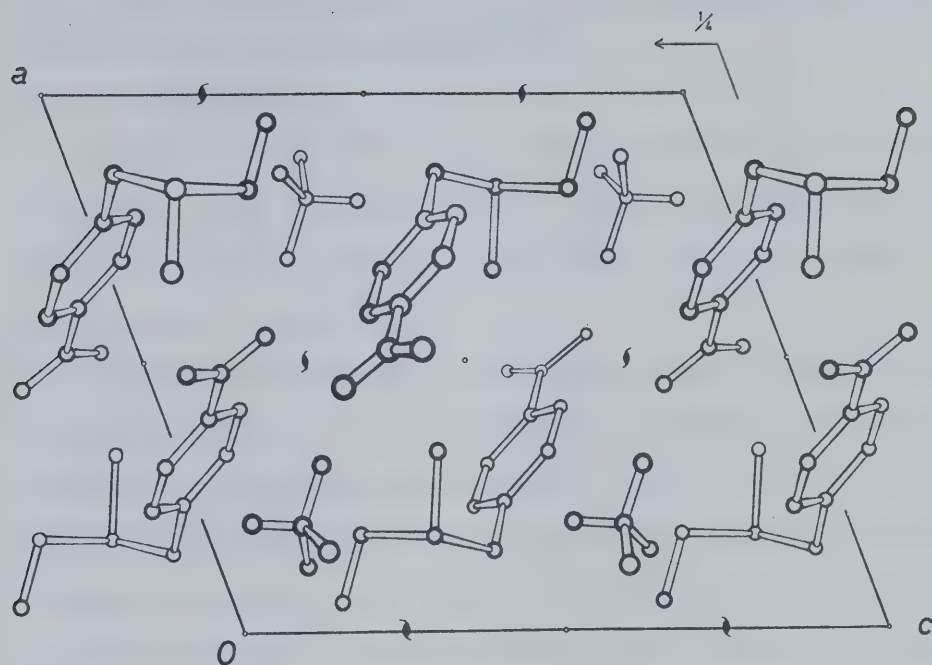


Figure 3.2.4 A projection of the unit cell onto  $(0\ 1\ 0)$ .



### 3.3 THE CRYSTAL AND MOLECULAR STRUCTURE OF (L)-3,5-DIMETHYL-4-METHOXY-BENZYLETHYLMETHYLSULFONIUM PERCHLORATE

#### 3.3.1 Experimental

Laevorotatory crystals of the title compound were obtained from Anita Po. Vapor diffusion of diethyl ether into a cold methanolic solution of the sulfonium salt yielded hard lustrous colorless prisms with 2 2 2 morphology.

Preliminary Weissenberg and precession photographs showed mm diffraction symmetry with the odd-ordered reflections for each of the three principal axes systematically unobserved. The crystals are thus orthorhombic with space group  $P2_1^2 2_1^2 2_1^2$ . Several prominent reflections having a small Bragg angle showed evidence of diffuse thermal scattering. The diffractivity of the crystals increased after exposure to liquid nitrogen temperatures for several seconds. This procedure did not affect the physical appearance of the crystals and was performed for all crystals used in the collection of intensity data.

A crystal specimen measuring 0.3 x 0.2 x 0.45 mm. (a x b x c) was mounted with the c-axis of the crystal parallel to the diffractometer  $\phi$ -shaft and used in the initial alignment of the machine. Cell dimensions were obtained from the angular coordinates of 12 reflections by the method of Busing and Levy (33). Unit cell data and physical constants are listed in Table 3.3.1.

Intensity data were obtained with the previously described crystal for all reflections in one quadrant of reciprocal space and  $3^\circ < 2\theta < 120^\circ$  using Ni-filtered Cu K radiation. All reflections were measured by the  $\theta/2\theta$  moving crystal/moving detector method at



Table 3.3.1

A summary of data for the crystal structure of 3,5-dimethyl-4-methoxybenzylethylmethyl-sulfonium perchlorate.

(a) Physical constants and unit cell data.

|                        |  |
|------------------------|--|
| Formula                | $C_{13}H_{21}O_5SCl$   |
| Molecular weight       | 324.8  |
| Melting point (85)     | 111-112°C  |
| Specific rotation (85) | $[\alpha]_{589}^{25} = -14.1^\circ$ (methanol)                         |
| Space group            | $P2_1^2 2_1^2 2_1^2$   |
| Cell dimensions        | $a = 7.486(1) \text{ \AA}$<br>$b = 24.679(3)$<br>$c = 8.639(1)$        |
| Volume                 | $V = 1596.0 \text{ \AA}^3$   |
| Density                | observed $1.35(1) \text{ g/cm}^3$<br>calculated $1.352 \text{ g/cm}^3$ |
| Cell content           | $z = 4$ molecules/unit cell  |
| Absorption coefficient | $\mu = 34.3 \text{ cm}^{-1}$ (Cu $K\alpha$ )                           |

(b) Experimental data\*

|                                     |   |
|-------------------------------------|---|
| Specimen size (a x b x c)           | 0.16 x 0.13 x 0.19 mm                           |
| Radiation                           | graphite monochromatized Cu $K\alpha$           |
| Scan mode                           | $\theta/2\theta$ moving crystal/moving detector |
| Scan rate                           | $2^\circ 2\theta/\text{min}$ .                  |
| Region of reciprocal space measured | $3^\circ < 2\theta < 129.8^\circ$               |
| No. of unique reflections measured  | 1602  |
| No. of reflections observed         | 1278  |
| Final residual R                    | 0.048   |
| Final weighted residual $R_w$       | 0.062   |

\* This data pertains to the second crystal used in the collection of intensity data.



a rate of  $1^\circ 2\theta/\text{min}$  and a scan width  $1.45^\circ 2\theta$  modified for the dispersion of the  $\alpha_1\alpha_2$  doublet. Stationary backgrounds were counted for 20 seconds at the  $2\theta$  boundaries of each reflection. Three standard reflections were collected every 33 intensity measurements to monitor the data for intensity loss due to crystal slippage and/or decomposition. During the data collection 2978 intensity measurements were performed and the net counts of the 3 standard reflections were found to decrease by an average of 16.6% of their initial values.

The reflection intensities were scaled and corrected for decomposition and Lorentz and polarization effects. An observational weight was calculated for each reflection. Only the data for 1421 unique reflections from the  $hkl$  octant were used in the solution and refinement of the structure; 1292 of these had  $I > 3\sigma(I)$  and were considered observed.

A Wilson plot (36) using these intensities produced a value of  $4.79 \text{ \AA}^2$  for the overall temperature factor and 0.3504 for the scale factor. A sharpened Patterson map was calculated in which the coefficients  $|F|_{\text{sharp}}^2$  were obtained from the expression

$$|F|_{\text{sharp}}^2 = |F_o|^2 \times \left\{ \frac{1 + \cos^2 2\theta}{2 \sin 2\theta} \right\}$$

The sharpening factor in parentheses corresponds to the inverse of the correction curve for Lorentz and polarization effects and  $\theta$  is the Bragg angle of the reflection.

The largest peaks on each of the three Harker sections formed





a self-consistent set and were tentatively identified as the sulfur-sulfur vectors. The Harker vectors corresponding to the remaining heavy atom were not identified due to insufficient sharpening in the Patterson synthesis.

The value of the agreement index for the structure factor calculation using the 'sulfur' coordinates  $x/a=0.108$ ,  $y/b=0.067$  and  $z/c=0.175$  and the values of the scale and overall temperature factor determined from the Wilson plot was 0.54. A heavy atom Fourier using the calculated phases of this set of structure factors revealed the locations of nine atoms. The set of atomic coordinates determined from the Patterson synthesis corresponded to the chlorine atom. A second Fourier map which was phased on sulfur, chlorine and seven carbon atoms successfully determined the locations of the remaining non-hydrogen atoms. The value of the residual using the unrefined set of parameters for 20 atoms, a scale of 0.3504 and an overall temperature factor  $B=4.76 \text{ \AA}^2$  was 0.403.

The atomic parameters were refined by the method of full-matrix least-squares using a locally modified version of ORFLS (98). The function minimized was  $D = \sum w ||F_o| - |F_c|^2 = \sum w \Delta^2$  where  $w$  is the weight given to each reflection.

The refinement was allowed to proceed in a stepwise manner with only the scale and the positional parameters refining during the first six cycles of least-squares. All reflections were given a weight of 1.0 with each atom having an isotropic thermal parameter of  $4.0 \text{ \AA}^2$ . This procedure decreased the residual to 0.204 and was followed by four cycles of least-squares in which both the positional and thermal parameters for 20 atoms were refined. This resulted in convergence of the atomic parameters at a residual of 0.128.



A difference Fourier calculated at that stage of the refinement using only the reflection data with  $\sin^2 \theta < 0.5$  showed nineteen maxima with values between 0.45 and 0.25  $e/\text{\AA}^3$ . These were tentatively identified as the hydrogen atoms, assigned the isotropic thermal parameters of the bonded carbon atom and included in subsequent structure factor calculations.

The refinement was continued for an additional three cycles using an anisotropic thermal model for the non-hydrogen atoms and keeping the parameters of the hydrogen atoms constant. The values of the agreement index for the three least-squares cycles were 0.125, 0.110 and 0.109. A difference Fourier calculated at the lowest residual showed irregular regions of positive and negative difference density in the vicinity of the phenyl and perchlorate moieties. It was decided that further refinement of the structure would not result in an improved agreement due to the adverse effects of crystal decomposition before and during the data collection.

A second crystal measuring 0.16 x 0.13 x 0.19 mm (a x b x c) was aligned on the FACS-1 diffractometer and used for the collection of intensity data for all reflections in one octant of reciprocal space and  $2\theta < 129.8^\circ$  with graphite monochromatized Cu K $\alpha$  radiation. A total of 1796 reflections were scanned by the  $\theta/2\theta$  moving crystal/moving detector method at a rate of  $2^\circ 2\theta/\text{min.}$  for a reflection width of  $1.5(2\theta)$  modified for dispersion of the  $\alpha_1\alpha_2$  doublet. Backgrounds were counted for 10 sec. at each of the reflection boundaries. Three standard reflections were monitored every 33 intensity measurements. During the data collection the average decrease in the intensities of the standard reflections was 3.8%



of the initial values. A total of 1602 unique reflections were recorded of which 1278 satisfied the criterion  $I > 3\sigma(I)$ . The intensity data were corrected for decomposition, Lorentz and polarization effects and an observational weight was calculated for each reflection.

The refinement was restarted using the non-hydrogen atomic parameters corresponding to the residual 0.128 with a value of 2.728 for the scale. This was estimated from the ratio between the values of  $|F_o|$  for several reflections in each data set. After 4 cycles of least-squares in which the atomic parameters were refined using an isotropic thermal model and observational weights, the refinement was continued for 2 cycles with the program NRC-10 (employing the block diagonal approximation) allowing the thermal parameters to adjust with an anisotropic model.

A difference Fourier at the residual 0.075 revealed the locations of 18 hydrogen atoms. In addition, four maxima with values of 0.78, 0.61, 0.52 and  $0.48 \text{ e/\AA}^3$  were tetrahedrally disposed about the chlorine atom at approximate C1-O bonding distances. This suggested the perchlorate ion was thermally disordered. The newly found oxygen atoms were assigned isotropic thermal parameters  $B=6.0\text{\AA}^2$  and occupancy factors of 0.125. The occupancy factors for the major site oxygen atoms were left at 1.0 although this represented a physically unreal situation. The hydrogen atoms were given the isotropic thermal parameter of the bonded carbon atom corresponding to the residual 0.091. The newly found atoms were included in the structure factor calculation and the remaining three hydrogen atom positions were located from a second difference Fourier. This procedure



lowered the residual to 0.070. The parameters of the hydrogen atoms were not refined subsequently. Several cycles of least-squares allowing the occupational parameters of the perchlorate oxygen atoms to refine resulted in a decrease of the residual to  $R=0.062$  but also in a physically unmeaningful description for the perchlorate. Two of the minor site oxygen atoms were found to have  $B < 1.5 \text{ \AA}^2$  with negative occupancy factors.

To eliminate this problem, each of the perchlorate oxygen atoms was assigned an isotropic thermal parameter of  $B=6.0 \text{ \AA}^2$ . The occupancy factors corresponding to the two sites were set at 1.0 and 0.125.

The parameters of the 8 disordered atoms were refined by the method of full-matrix least-squares for 4 cycles using an isotropic thermal model followed by 2 cycles using an anisotropic thermal model. This process decreased the residual to 0.054 and resulted in average values of 0.73 and 0.32 for the occupancy factors of the two perchlorate tetrahedral sites.

The refinement was continued for 4 cycles allowing all of the non-hydrogen atomic parameters to adjust. This resulted in the convergence of the parameters for all but the perchlorate oxygen atoms at the residual  $R=0.048$ . The thermal parameters and occupational factors for the disordered atoms were still oscillating and it was decided that further refinement would not be worthwhile in view of the high cost of computing. The refinement was therefore terminated. The final values of the weighted and unweighted residuals were 0.062 and 0.048 respectively.





Table 3.3.2

The final positional parameters ( $\times 10^4$ ) for the non-hydrogen atoms of the 3,5-dimethyl-4-methoxybenzylethylmethylsulfonium cation. The e.s.d's are included in parenthesis.

| Atom  | x/a      | y/b     | z/c      |
|-------|----------|---------|----------|
| C(1)  | 8911(1)  | 9385(2) | 8176(3)  |
| S(1)  | 6038(2)  | 5730(1) | 6570(2)  |
| C(1)  | 5940(11) | 5030(2) | 7020(12) |
| C(2)  | 7152(9)  | 6012(2) | 8248(9)  |
| C(3)  | 9169(11) | 5926(3) | 8134(12) |
| C(4)  | 3766(8)  | 5932(2) | 6941(7)  |
| C(5)  | 3540(7)  | 6537(2) | 6755(6)  |
| C(6)  | 3642(8)  | 6767(2) | 5315(6)  |
| C(7)  | 3452(7)  | 7327(2) | 5106(7)  |
| C(8)  | 3148(8)  | 7640(2) | 6419(7)  |
| C(9)  | 3031(8)  | 7421(2) | 7889(7)  |
| C(10) | 3284(8)  | 6863(2) | 8040(6)  |
| C(11) | 3418(10) | 7557(3) | 3508(8)  |
| O(12) | 2847(6)  | 8183(2) | 6189(5)  |
| C(13) | 4259(11) | 8540(3) | 6563(10) |
| C(14) | 2592(11) | 7766(3) | 9276(7)  |



Table 3.3.3

The final positional and thermal parameters ( $\times 10^3$ )  
for the hydrogen atoms of (L)-3,5-dimethyl-4-methoxy-  
benzylethylmethylsulfonium perchlorate.

| Atom   | x/a | y/b | z/c | $U_{iso}$ |
|--------|-----|-----|-----|-----------|
| H(11)  | 560 | 500 | 809 | 827       |
| H(12)  | 500 | 489 | 656 | 827       |
| H(13)  | 706 | 487 | 694 | 827       |
| H(21)  | 668 | 583 | 922 | 671       |
| H(22)  | 689 | 642 | 831 | 671       |
| H(31)  | 963 | 615 | 869 | 807       |
| H(32)  | 954 | 597 | 715 | 807       |
| H(33)  | 946 | 559 | 844 | 807       |
| H(41)  | 343 | 582 | 804 | 600       |
| H(42)  | 294 | 574 | 619 | 600       |
| H(61)  | 392 | 655 | 445 | 505       |
| H(101) | 328 | 670 | 904 | 553       |
| H(111) | 230 | 771 | 310 | 698       |
| H(112) | 411 | 777 | 369 | 698       |
| H(113) | 373 | 727 | 282 | 698       |
| H(131) | 455 | 846 | 738 | 749       |
| H(132) | 534 | 833 | 576 | 749       |
| H(133) | 386 | 892 | 644 | 749       |
| H(141) | 344 | 791 | 969 | 781       |
| H(142) | 205 | 752 | 999 | 781       |
| H(143) | 185 | 806 | 893 | 781       |



Table 3.3.4

The final anisotropic thermal parameters ( $\times 10^4$ )  
for the atoms listed in Table 3.3.2. Parameter  
e.s.d's are included in parentheses.

| Atom  | $U_{11}^*$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{23}$ | $U_{13}$ |
|-------|------------|----------|----------|----------|----------|----------|
| C(1)  | 273(4)     | 262(4)   | 247(3)   | 38(3)    | -5(3)    | -20(4)   |
| S(1)  | 247(4)     | 249(4)   | 278(4)   | 15(3)    | -18(3)   | 17(4)    |
| C(1)  | 341(19)    | 213(13)  | 613(32)  | 9(16)    | -38(17)  | -2(26)   |
| C(2)  | 267(16)    | 247(14)  | 347(19)  | -24(13)  | -46(15)  | -26(17)  |
| C(3)  | 306(20)    | 313(17)  | 602(31)  | 20(16)   | -68(20)  | -151(25) |
| C(4)  | 228(15)    | 225(12)  | 268(14)  | -18(12)  | -22(11)  | -13(15)  |
| C(5)  | 165(12)    | 219(10)  | 209(12)  | 6(12)    | 11(10)   | 11(11)   |
| C(6)  | 200(2)     | 234(12)  | 203(12)  | 33(12)   | -40(10)  | -10(12)  |
| C(7)  | 167(12)    | 284(16)  | 233(13)  | -8(12)   | 5(12)    | 6(12)    |
| C(8)  | 175(13)    | 235(13)  | 252(15)  | 14(11)   | -15(11)  | -47(12)  |
| C(9)  | 218(15)    | 251(13)  | 232(15)  | 38(12)   | -63(11)  | -17(12)  |
| C(10) | 219(14)    | 292(14)  | 190(12)  | 16(10)   | 0(11)    | -10(12)  |
| C(11) | 302(21)    | 366(18)  | 250(17)  | -8(15)   | 17(15)   | -23(16)  |
| O(12) | 270(12)    | 231(9)   | 385(14)  | 53(9)    | -11(9)   | -70(11)  |
| C(13) | 332(20)    | 273(16)  | 523(28)  | -4(16)   | -6(18)   | -30(24)  |
| C(14) | 330(22)    | 432(22)  | 237(16)  | 67(18)   | -50(15)  | -23(16)  |

\*These coefficients are defined in the Preface



Table 3.3.5

The final positional parameters ( $\times 10^3$ ) and occupancy factors for the disordered perchlorate oxygen atoms. Parameters e.s.d.'s are included in parentheses.

| Atom | x/a      | y/b     | z/c    | occupancy factor* |
|------|----------|---------|--------|-------------------|
| O(1) | 940(3)   | 981(1)  | 918(2) | 0.755(69)         |
| O(2) | 1049(2)  | 912(1)  | 808(2) | 0.759(53)         |
| O(3) | 750(3)   | 910(1)  | 884(2) | 0.707(58)         |
| O(4) | 846(6)   | 960(1)  | 676(3) | 0.738(107)        |
| O(5) | 900(71)  | 886(12) | 858(3) | 0.412(71)         |
| O(6) | 818(21)  | 967(3)  | 925(5) | 0.372(79)         |
| O(7) | 762(20)  | 939(5)  | 693(9) | 0.224(112)        |
| O(8) | 1012(12) | 948(2)  | 682(5) | 0.223(47)         |

\* The e.s.d.'s for the occupancy factors are from the final cycle in which the occupancy factors were refined.

Table 3.3.6

The final thermal parameters ( $\times 10^3$ ) for the eight oxygen atoms listed in Table 3.3.5. Parameter e.s.d.'s are included in parentheses.

| Atom | $U_{11}^*$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{23}$ | $U_{13}$ |
|------|------------|----------|----------|----------|----------|----------|
| O(1) | 101(8)     | 44(4)    | 32(3)    | -14(6)   | -10(2)   | -8(3)    |
| O(2) | 42(5)      | 105(10)  | 70(7)    | 39(5)    | -4(7)    | -10(5)   |
| O(3) | 47(6)      | 77(13)   | 95(8)    | -21(6)   | 36(7)    | 0(5)     |
| O(4) | 74(14)     | 51(4)    | 25(4)    | -7(7)    | 10(4)    | -10(6)   |
| O(5) | 156(46)    | 38(6)    | 41(6)    | 21(10)   | 5(4)     | -20(12)  |
| O(6) | 258(51)    | 63(22)   | 60(11)   | 111(26)  | -9(12)   | -13(18)  |
| O(7) | 74(29)     | 53(20)   | 58(14)   | 30(20)   | -26(12)  | -44(14)  |
| O(8) | 62(26)     | 47(13)   | 40(13)   | -14(13)  | -13(10)  | 4(14)    |

\* These coefficients are defined in the Preface







Table 3.3.7

The observed structure amplitudes and final structure factors on the absolute scale  $\times 10$  for (L)-3,5-dimethyl-4-methoxybenzylethylmethysulfonium perchlorate. The unobserved reflections are designated by an asterisk (\*).

[illegible]







The final positional and thermal parameters for chlorine and the atoms of the cation are listed in Tables 3.3.2, 3.3.3 and 3.3.4; the final parameters for the perchlorate oxygen atoms are listed in Tables 3.3.5 and 3.3.6. Observed and calculated structure factors are listed on the absolute scale  $\times 10$  in Table 3.3.7.

### 3.3.2 Determination of the Absolute Configuration

In order to determine the absolute configuration, it was decided to compare the observed and calculated intensities of a number of Friedel pairs which would be expected to show unequal intensities due to the breakdown of Friedel's law in the presence of the anomalous scattering of sulfur and chlorine. Reflections were chosen for this purpose using the following criteria.

- (i) All reflections must have indices  $hkl$  such that
 
$$h \times k \times l \neq 0.$$
- (ii) The observed reflection intensity should be large enough to permit a statistically reliable measurement but not large enough to cause concern over possible effects of counter paralysis or extinction.
- (iii) The contribution of the chlorine and sulfur form factors to the overall structure factor should be large enough for the  $\Delta f_s''$  and  $\Delta f_{Cl}''$  terms to be observable.

A total of 165 reflections were selected with  $9.0 < F_o < 65.0$  and  $F_c(Cl) + F_c(S) > 0.25 F_c(all)$ .

A small crystal  $0.2 \times 0.18 \times 0.2$  mm showing almost perfect  $m\bar{2}m$  symmetry and no general faces, was immersed in liquid nitrogen for several seconds. The 165  $hkl/\bar{h}k\bar{l}$  pairs were collected







Table 3.3.8

Observed and calculated structure factor amplitudes for the  $hkl/\bar{h}k\bar{l}$  Friedel pairs used to determine the absolute configuration of (*l*)-3,5-dimethyl-4-methoxybenzyl-ethylmethysulfonium perchlorate.





and processed in the normal way on the FACS-1 diffractometer with graphite monochromatized Cu K $\alpha$  radiation. The data were corrected for decomposition, Lorentz and polarization effects. Absorption corrections were not applied.

A second set of atomic parameters was obtained by inversion of the  $R=0.048$  parameters through the origin of the unit cell. Two structure factor calculations were calculated using the R and S parameter sets and allowing only the scale factor to refine. When the  $\Delta f''_{Cl}$  (0.7 electrons) and  $\Delta f''_S$  (0.6 electrons) terms were included in the calculations the absolute configuration was determined to correspond to the R parameter set. The agreement summary for the two calculations is:

| Model | R     | $R_w$ |
|-------|-------|-------|
| R     | 0.034 | 0.054 |
| S     | 0.050 | 0.075 |

A tabulation of the observed and calculated structure factors for the R parameter set is given in Table 3.3.8.

In all but 12 cases the observed and calculated  $|F(h)| - |F(\bar{h})|$  differences agree with regard to sign. In each of the 12 exceptions, the relationship  $(|F_o(h)| - |F_o(\bar{h})|) - (|F_c(h)| - |F_c(\bar{h})|) \geq \sigma(F_o(h))$  is true.

### 3.3.3 Discussion and Results

This study has shown the laevorotatory 3,5-dimethyl-4-methoxy-benzylethylmethylsulfonium cation to have the R configuration as depicted in Figure 3.3.1. The conformation of the cation is



dissimilar to that observed for the p-nitro homologue (see Figure 3.2.1). The C(4)-C(5) bond is trans with respect to the C(1)-S bond; the C(4)-S bond is trans with respect to the C(2)-C(3) bond. In order to make the two conformations agree, the C(4)-C(5) vector should be rotated  $90^\circ$  about the S-C(4) bond followed by a  $-90^\circ$  rotation of the C(2)-C(3) vector about the S-C(2) bond.

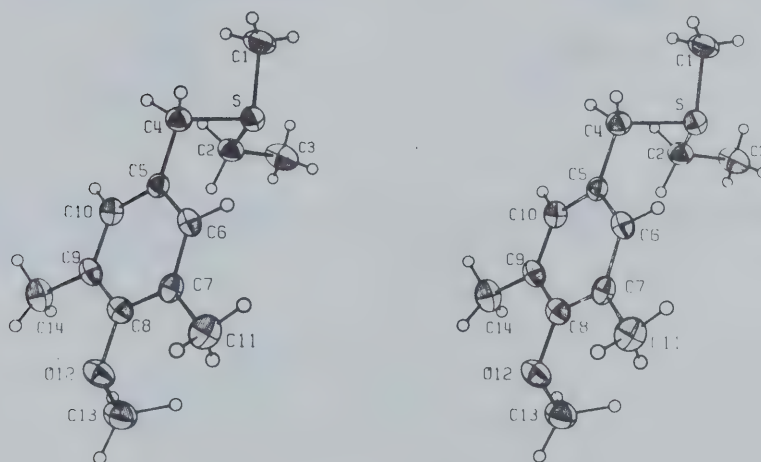


Figure 3.3.1 The molecular conformation of the 3,5-dimethyl-4-methoxybenzylethylmethylsulfonium cation. The anisotropic thermal ellipsoids are scaled to include 25% probability.

Bond distances and valence bond angles observed in the sulfonium cation are illustrated in Figure 3.3.2. Denoting C and O by X, the average e.s.d.'s in the angles SXX, XSX and XXX are 0.4, 0.3 and  $0.5^\circ$  respectively. Carbon-hydrogen bond distances are listed in Table 3.3.9. Interbond angles including hydrogen atoms range from 93 to  $124^\circ$  and are not listed.





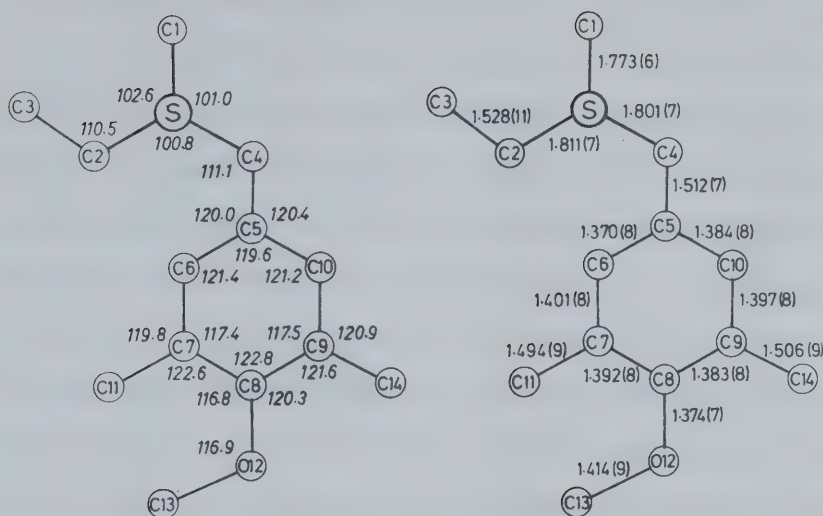


Figure 3.3.2 Bond lengths and valence bond angles (excluding hydrogen atoms) observed in the  $C_{13}H_{21}OS$  cation.

Table 3.3.9

### Carbon-Hydrogen Bond Distances

|            |        |              |        |
|------------|--------|--------------|--------|
| C(1)-H(11) | 0.96 Å | C(10)-H(101) | 0.95 Å |
| C(1)-H(12) | 0.88   | C(11)-H(111) | 0.98   |
| C(1)-H(13) | 0.93   | C(11)-H(112) | 0.76   |
| C(2)-H(21) | 1.02   | C(11)-H(113) | 0.96   |
| C(2)-H(22) | 1.02   | C(13)-H(131) | 0.76   |
| C(3)-H(31) | 0.80   | C(13)-H(132) | 1.19   |
| C(3)-H(32) | 0.90   | C(13)-H(133) | 0.99   |
| C(3)-H(33) | 0.90   | C(14)-H(141) | 0.81   |
| C(4)-H(41) | 1.02   | C(14)-H(142) | 0.96   |
| C(4)-H(42) | 1.02   | C(14)-H(143) | 0.96   |
| C(6)-H(61) | 0.94   |              |        |

$$\langle C-H \rangle = 0.94 \text{ Å}$$



The three carbon-sulfur bond distances corrected for thermal motion by the method of Busing and Levy (49) are listed in Table 3.3.10. An examination of the vibration ellipsoid for C(1) in Figure 3.3.1 suggests the motion of C(1), relative to sulfur is probably best described by the 'riding motion' approximation. Assuming that the corrections to the S-C(2) and S-C(4) bond lengths for thermal motion are intermediate between the lower bound and the 'riding motion' approximation, the corrected values of the S-C(1), S-C(2) and S-C(4) bond lengths are 1.800(6), 1.815(7) and 1.803(7) Å<sup>o</sup> respectively. The average C-S bond length is therefore 1.806 Å<sup>o</sup>.

Table 3.3.10

| Correction of the C-S bond<br>lengths for thermal motion |        |                            |                      |                      |                      |
|--|--------|----------------------------|----------------------|----------------------|----------------------|
| Atom A   | Atom B | Uncorrected<br>bond length | Lower<br>Bound       | Riding<br>Motion     | Upper<br>Bound       |
| S(1)   | C(1)   | 1.773(6) Å <sup>o</sup>    | 1.777 Å <sup>o</sup> | 1.800 Å <sup>o</sup> | 1.961 Å <sup>o</sup> |
| S(1)   | C(2)   | 1.811(7)                   | 1.811                | 1.818                | 1.956                |
| C(4)   | S(1)   | 1.801(7)                   | 1.801                | 1.805                | 1.936                |

The C(1)-S-C(2), C(1)-S-C(4) and C(2)-S-C(4) angles are 102.6, 101.0 and 100.8° with a simple average value of 101.5°. These three angles show approximately  $\frac{1}{2}$  the spread observed in the 4-nitrobenzyl homologue. The extended conformation of the cation reduces the number and magnitude of non-bonding interactions between the various substituents bonded to sulfur. In contrast to the previous structure, the relative absence of steric interactions between the phenyl ring



and the two alkyl substituents permits a regular value of  $111.1^\circ$  for the angle S-C(4)-C(5) which is equivalent to the S-C(2)-C(3) angle of  $110.5^\circ$ . The trans disposition of the three ligands to sulfur allows the sulfonium pole to have a more prominent pyramidal shape. The equation of the plane defined by the three  $\alpha$ -carbon atoms is  $0.3777x + 0.2489y - 0.8918z = -1.9047$ . The distance of the sulfur atom from this plane is  $0.804(1) \text{ \AA}$ .

The C(sp<sup>2</sup>)-C(sp<sup>2</sup>) bond distances range from  $1.401(8)$  to  $1.370(8) \text{ \AA}$  with a mean value of  $1.388 \text{ \AA}$ ; the standard deviation of the mean is  $0.005 \text{ \AA}$ . The three C(sp<sup>3</sup>)-C(sp<sup>2</sup>) distances of  $1.512(7) \text{ \AA}$ ,  $1.506(9) \text{ \AA}$  and  $1.494(9) \text{ \AA}$  concur with the average C(sp<sup>3</sup>)-C(sp<sup>2</sup>) bond length of  $1.505(5) \text{ \AA}$  listed by Sutton (6). The bond angles in the phenyl ring are symmetrical about the C(5)-C(8) vector with one exception. The C(9)-C(8)-O(12) angle of  $120.3^\circ$  is significantly larger than the C(7)-C(8)-O(12) angle of  $116.8^\circ$ . The inequality of the two angles is consistent with the differences between the short non-bonded contacts C(14)···C(13) ( $3.27 \text{ \AA}$ ) and C(11)···C(13) ( $3.64 \text{ \AA}$ ). The larger values of the angles C(11)-C(7)-C(8) ( $122.6^\circ$ ), C(7)-C(8)-C(9) ( $122.8^\circ$ ) and C(8)-C(9)-C(14) ( $121.6^\circ$ ) are probably due to Van der Waals repulsions between the methoxy substituent and the two ring methyl substituents.

In contrast to the 4 aromatic methyl ethers (103-105) whose structural parameters are listed in Table 3.3.11, the O-methyl substituent is not coplanar with the phenyl ring. Steric interactions between the C(7)- and C(9)-methyl substituents and the methoxy group push all three substituents out of the ring plane. The C(8)-O(12)-C(13) angle of  $116.9^\circ$ , while typical of similar



Table 3.1.11

Bond distances and angles observed in several structures containing an aromatic methyl ether.

| Compound  | Bond<br>CH <sub>3</sub> -O | Distances<br>O-C(sp <sup>2</sup> ) | Angle<br>CH <sub>3</sub> -O-C(sp <sup>2</sup> ) | Exoplanar |                 |
|---|----------------------------|------------------------------------|---|-----------|-----------------|
|   |                            |                                    |   | O         | CH <sub>3</sub> |
| desoxyanisoin (103)   | 1.432(10) Å                | 1.374(10) Å                        | 116.3(6)°                                       | 0.02 Å    | 0.13 Å          |
|   | 1.435(10)                  | 1.365(10)                          | 117.5(6)  | -0.02     | -0.11           |
| p,p'-dimethoxybenzophenone (103)                                  | 1.426(10)                  | 1.350(10)                          | 118.9(6)  | 0.01      | 0.01            |
|   | 1.422(10)                  | 1.368(10)                          | 118.3(6)  | 0.03      | -0.02           |
|   | 1.419(10)                  | 1.359(10)                          | 118.0(6)  | 0.05      | 0.03            |
|   | 1.428(10)                  | 1.373(10)                          | 118.0(6)  | 0.02      | 0.05            |
| codeine hydrobromide dihydrate (104)                              | 1.443(21)                  | 1.399(21)                          | 117.2(1.2)                                      | 0.16      | 0.32            |
| 5-methoxy-(N,N)-dimethyltrypt-<br>amine (105)                     | 1.419(5)                   | 1.363(3)                           | 118.3(3)  | 0.00      | 0.08            |
| 3,5-dimethyl-4-methoxybenzylethyl-<br>methylsulfonium perchlorate | 1.414(9)                   | 1.374(7)                           | 116.9(5)  | 0.08      | -1.10           |





angles listed in Table 3.3.11, is nevertheless, larger than the angles observed in aliphatic ethers (i.e. diethyl ether (106), 111.7 and 112.0(3)<sup>o</sup>). This is probably due to a Van der Waals interaction between the C(13)-methyl group and the  $\pi$ -electron cloud of the phenyl ring.

A best least-squares fit of the positional parameters of the six aromatic carbon atoms to the direction-cosine equation of a plane gave the equation  $-0.9866x - 0.1215y - 0.1088z = 6.1047$  ( $\chi^2 = 21.5$ ). The exoplanar distances of the aromatic carbon atoms are C(5), 0.010; C(6), 0.001; C(7), -0.007; C(8), 0.000; C(9), 0.014 and C(10), -0.020 Å. The exoplanar distances of substituents bonded to the ring are C(4), 0.007; C(11), 0.099; O(12), 0.081; C(14), 0.105; H(61), -0.061; H(101), -0.064 and C(13), -1.104 Å. C(11), O(12) and C(14) are on one side of the phenyl ring plane, C(13) is displaced towards the opposite side of the phenyl ring.

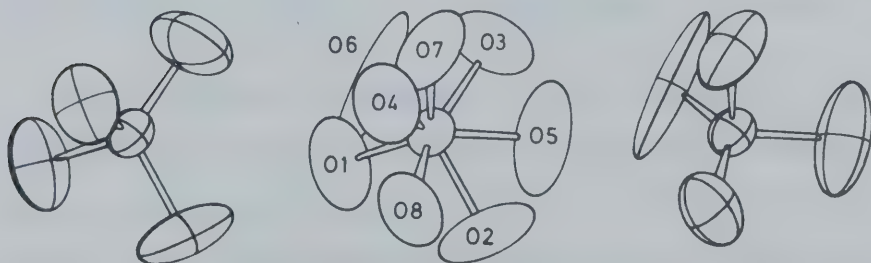


Figure 3.3.3 The disordered perchlorate anion. The anion is partitioned into its major and minor tetrahedral components. The thermal ellipsoids of vibration are scaled to include 25% probability. No simple symmetry relationship exists between the two sets of oxygen atoms.



Table 3.3.12

## Chlorine-oxygen bond distances

|         |           |         |            |
|---------|-----------|---------|------------|
| Cl-O(1) | 1.41(2) Å | Cl-O(5) | 1.35(29) Å |
| Cl-O(2) | 1.36(2)   | Cl-O(6) | 1.28(9)    |
| Cl-O(3) | 1.40(2)   | Cl-O(7) | 1.45(11)   |
| Cl-O(4) | 1.37(3)   | Cl-O(8) | 1.50(6)    |

Table 3.3.13

Interbond angles observed within  
the perchlorate tetrahedra de-  
picted in Figure 3.3.3.

| Ordered Tetrahedron | Angle   | Disordered Tetrahedron | Angle    |
|---------------------|---------|------------------------|----------|
| O(1)-Cl-O(2)        | 100(1)° | O(5)-Cl-O(6)           | 112(15)° |
| O(1)-Cl-O(3)        | 109(1)  | O(5)-Cl-O(7)           | 104(16)  |
| O(1)-Cl-O(4)        | 109(2)  | O(5)-Cl-O(8)           | 109(15)  |
| O(2)-Cl-O(3)        | 116(1)  | O(6)-Cl-O(7)           | 104(7)   |
| O(2)-Cl-O(4)        | 110(2)  | O(6)-Cl-O(8)           | 137(5)   |
| O(3)-Cl-O(4)        | 112(2)  | O(7)-Cl-O(8)           | 80(5)    |

Chlorine-oxygen bond distances are listed in Table 3.3.12. None of these distances were corrected for thermal motion due to the poor precision of each value. Whereas the uncorrected Cl-O distances within the tetrahedron defined by the oxygen atoms O(1) to O(4) are not significantly different from each other, the remaining four Cl-O distances exhibit a wide variation which is not surprising if one considers the interaction of the occupancy factor and thermal parameters for each oxygen 'atom'. The O-Cl-O



angles for each tetrahedron are listed in Table 3.3.13. Again, a wide range of angles is observed for the tetrahedron having the oxygen atoms with the lower occupancy factors. Although the sum of the occupancy factors (see Table 3.3.6) for the eight oxygen atoms is 4.19, the average percentage error in the occupancy factors is 18.5%.

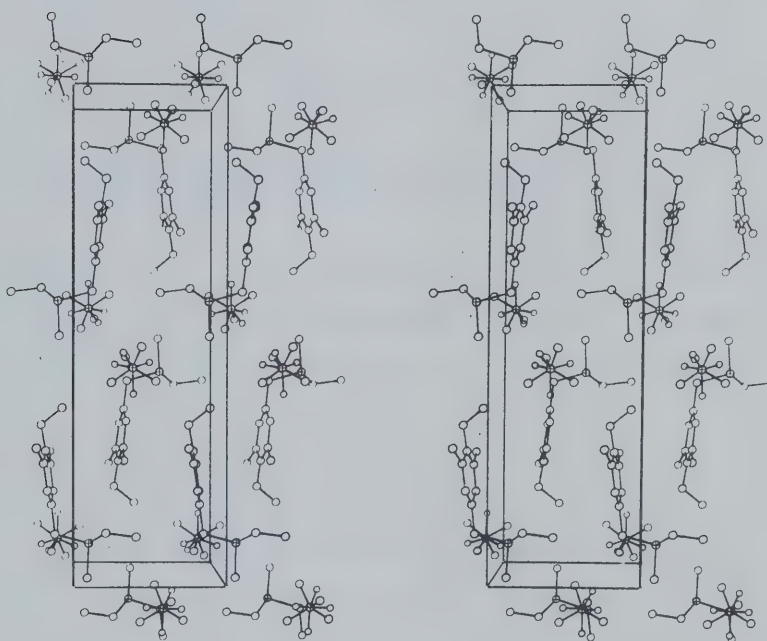


Figure 3.3.4 The arrangement of the perchlorate anions and sulfonium cations within the unit cell viewed along the z-axis. The origin of the unit cell corresponds to the upper left hand corner of the enclosure. The x-axis points towards the right hand side, the y-axis points towards the bottom of the page.



The spatial arrangement of the sulfonium cations and perchlorate anions in the unit cell is shown in Figure 3.3.4. The lattice is held together in the x- and z-directions by a series of alternating positive and negative charges. Figure 3.3.4 clearly shows the disposition of the eight oxygen 'atoms' of the perchlorate ion. The oxygen atoms O(1) to O(4) are depicted by large spheres; O(5) to O(8) are depicted by small spheres. Sulfur...oxygen contacts less than  $3.9 \text{ \AA}$  are listed in Table 3.3.14.

Table 3.3.14

Sulfur...oxygen contacts less than  $3.9 \text{ \AA}$

|       |          |                    |
|-------|----------|--------------------|
| S(1)i | O(8) ii  | $3.05 \text{ \AA}$ |
| S(1)i | O(4) iii | 3.56               |
| S(1)i | O(12)ii  | 3.84               |

|     |                     |                     |          |
|-----|---------------------|---------------------|----------|
| i   | x,                  | y,                  | z        |
| ii  | $\frac{1}{2} + x,$  | $-\frac{1}{2} - y,$ | $-1 + z$ |
| iii | $-\frac{1}{2} - x,$ | $\frac{1}{2} + y,$  | $-1 + z$ |

Because only one short contact exists between the perchlorate ion and the positively charged sulfur atom, the perchlorate ion has a greater freedom to rotate or spin within the lattice compared to the perchlorate ion in the previous structure.





### 3.4 THE CRYSTAL AND MOLECULAR STRUCTURE OF (d)-4-CHLOROBENZYLETHYL-METHYLSULFONIUM PERCHLORATE

#### 3.4.1 Experimental

A sample of dextrorotatory 4-chlorobenzylethylmethysulfonium perchlorate was obtained from Anita Po. Elongated prisms exhibiting a diamond shaped cross-section and 2 2 2 morphology were obtained by the vapor diffusion of diethyl ether into a cold concentrated methanolic solution of the sulfonium salt. The crystals were strongly birefringent and gave conchoidal fracture. Preliminary Weissenberg and precession photographs indicated the crystal had mm diffraction symmetry with the odd-ordered reflections of the three principal axes systematically unobserved. Thus the space group is  $P2_1^2 2_1^2 2_1^2$ . During the characterization of the diffraction pattern, the distribution of intensities among the series of Weissenberg photographs  $hk0$ ,  $hk2$ ,  $hk4$  and  $hk6$  were found to be remarkably similar. This phenomenon was observed to a lesser degree among the films  $hk1$ ,  $hk3$  and  $hk5$ .

The unit cell parameters, obtained initially from films, were refined by a least-squares process (33) during the alignment of the crystal on the Picker FACS-1 diffractometer. The lattice parameters and other data pertaining to the collection of intensities are listed in Table 3.4.1.

Intensity measurements were performed on a crystal  $0.25 \times 0.2 \times 0.4$  mm (a x b x c) using graphite monochromatized Cu K $\alpha$  radiation at a take-off angle of  $5^\circ$ . Reflections were scanned at a rate of  $2^\circ 2\theta/\text{min.}$  with the diffractometer in the  $\theta/2\theta$  moving crystal/moving detector mode. Each reflection was given a standard scan width of



Table 3.4.1

A summary of experimental data for  
(d)-4-chlorobenzylethylmethysulfonium  
perchlorate.

|                                     |  |
|-------------------------------------|--|
| Molecular formula                   | $C_{10}H_{14}O_4SCl_2$   |
| Molecular weight                    | 301.2  |
| Melting point (85)                  | 88°C.  |
| Specific rotation (85)              | $[\alpha]_{436}^{25} = +31.5^\circ$ (methanol)   |
| Space group                         | $P2_1^2 2_1^2 2_1^2$   |
| Cell dimensions                     | $a = 12.307(2) \text{ \AA}$<br>$b = 13.736(3) \text{ \AA}$<br>$c = 7.998(2) \text{ \AA}$ |
| Volume                              | $V = 1352.1 \text{ \AA}^3$   |
| Density                             | observed $1.49(1) \text{ g/cm}^3$<br>calculated $1.479 \text{ g/cm}^3$                   |
| Linear absorption coefficient       | $\mu = 57.1 \text{ cm}^{-1}$ (Cu $K\alpha$ )   |
| Scan mode                           | $\theta/2\theta$ moving crystal/moving detector  |
| Scan rate                           | $2^\circ 2\theta/\text{min.}$  |
| Region of reciprocal space examined | $3^\circ < 2\theta < 129.3^\circ$  |
| No. of reflections measured         | 1351   |
| No. of reflections observed         | 1155   |
| Final conventional residual R       | 0.043  |
| Final weighted residual $R_w$       | 0.052  |



$1.5^\circ 2\theta$  which was modified to allow for the increased dispersion of the  $\alpha_1\alpha_2$  doublet as a function of  $\theta$ . Stationary backgrounds were counted for 10 sec. at the  $2\theta$  boundaries of each scan.

Crystal decomposition was monitored by the collection of three standard reflections once every 33 measurements. During the data collection 1351 lattice points were examined in one octant of reciprocal space with  $3^\circ < 2\theta < 129.3^\circ$ . The average decrease in the intensities of the standard reflections during the course of the data collection was less than 2% of the initial values.

The intensity data were scaled and corrected for decomposition and Lorentz and polarization effects (34). Reflections for which the relationship  $I > 3\sigma(I)$  was valid were considered to have intensities significant above background. The unobserved reflections were assigned a threshold intensity but excluded from all further calculations. An observational weight was calculated for each reflection using the equation listed in the Preface.

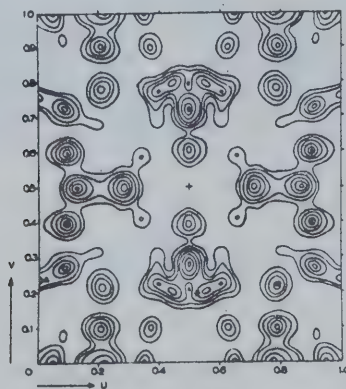


Figure 3.4.1 The Harker section ( $U, V, \frac{1}{P}$ ). The locations of the S-S, Cl(1)-Cl(1) and Cl(2)-Cl(2) vectors are indicated by a ▲, ■ and \* respectively.



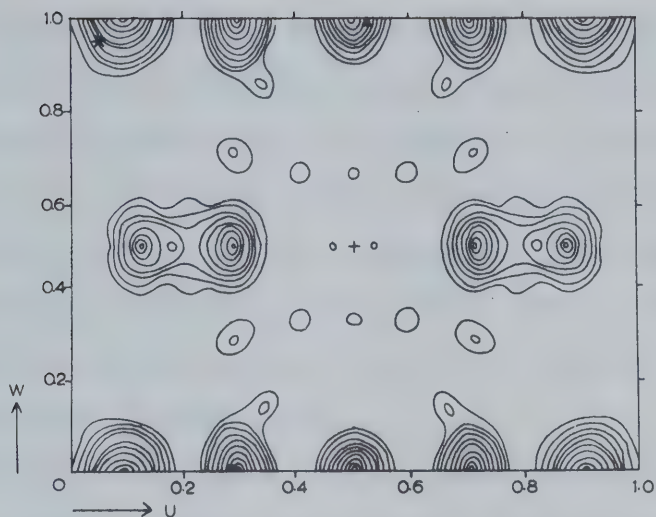


Figure 3.4.2 The Harker section ( $U, \frac{1}{2}, W$ ). The S-S,  $Cl(1)-Cl(1)$  and  $Cl(2)-Cl(2)$  vectors are indicated by a  $\blacktriangle$ ,  $\blacksquare$  and  $*$  respectively.

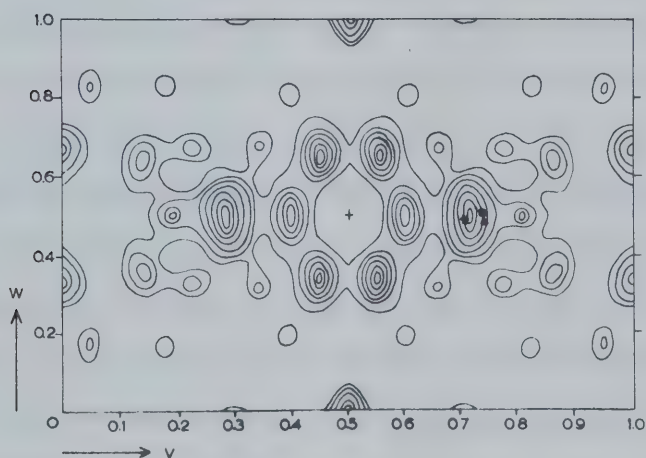


Figure 3.4.3 The Harker section ( $\frac{1}{2}, V, W$ ). The S-S,  $Cl(1)-Cl(1)$  and  $Cl(2)-Cl(2)$  vectors are indicated by a  $\blacktriangle$ ,  $\blacksquare$  and  $*$  respectively.





The three Harker sections  $(U, V, \frac{1}{2})$ ,  $(U, \frac{1}{2}, W)$  and  $(\frac{1}{2}, V, W)$  of a  $1/L_p$  sharpened Patterson synthesis are shown in Figures 3.4.1, 3.4.2 and 3.4.3. The Patterson map was characterized by a very large number of peaks on  $(U, V, 0)$  and  $(U, V, \frac{1}{2})$  and to a lesser extent  $(U, V, 1/6)$  and  $(U, V, 2/6)$ . The appearance of the Harker sections  $(\frac{1}{2}, V, W)$  and  $(U, \frac{1}{2}, W)$  suggested the  $z/c$  coordinates for the majority of atoms to be 0.25 and 0.75. This concurs with the high density of peaks on  $(U, V, 0)$  and  $(U, V, \frac{1}{2})$  which would include a large number of cross vectors.

To eliminate sets of cross vectors from the last Harker section, the orientation of the cation was determined in the following way. Structure factor graphs of the reflections  $(0\ 0\ 6)$ ,  $(12\ 7\ 0)$  and  $(0\ 10\ 4)$  were drawn to the scale  $1''/\text{\AA}$  and a scaled model of the sulfonium cation was simultaneously fitted to each graph so that all atoms would contribute to the structure factor with the same sign. The conformation of the cation was assumed to be similar to the 4-nitrobenzyl homologue. Only one orientation simultaneously agreed with these conditions and the vector distribution on each of  $(U, \frac{1}{2}, W)$  and  $(\frac{1}{2}, V, W)$ . The vector  $\text{Cl}(1)\text{-C}(4)$  was parallel to  $y$  with  $\text{C}(6)\text{-C}(10)$  parallel to  $z$  and  $\text{C}(4)\text{-S}(1)$  in the  $x\text{-}y$  plane. An inspection of the  $(0, V, W)$  section revealed the image of the phenyl ring and subsequently a large number of cross-vectors were eliminated from  $(U, V, \frac{1}{2})$ .

One regularly shaped self-consistent vector set was tentatively identified to be the chlorine-chlorine Harker vectors and yielded possible coordinates of  $x/a=0.144$ ,  $y/b=0.608$  and  $z/c=0.25$ . A symmetry minimum function (70) based on this position ( $\text{Cl}(1)$ )



using the  $1/L_p$ -sharpened Patterson synthesis yielded a set of peaks which were identified to represent the locations of the perchlorate and the chlorobenzyl-sulfur moieties. A second symmetry minimum function based on C(1) and C(7) ( $z/c \neq 0.25, 0.75$ ) yielded the same set of peaks. The locations of the three remaining carbon atoms were determined by Fourier methods using coefficients weighted according to the method of Sim (71). Although two peaks roughly symmetrical about  $z/c=0.25$  could be chosen for the location of the  $\beta$ -carbon of the ethyl substituent, the strongest peak was found to give the best agreement. The initial value of the residual for the unrefined parameters of 17 atoms assigning each an isotropic thermal parameter  $B=3.5 \text{ \AA}^2$  and a scale of 0.2911 (from a Wilson plot (36)) was 0.36.

Atomic parameters were refined by the method of least-squares using NRC-10 (35) which employs the block-diagonal approximation. The quantity minimized was  $D = \sum w ||F_o| - |F_c||^2$ . A full-matrix least-squares refinement was preferable but initially unavailable due to programming difficulties.

The positional and isotropic thermal parameters for 17 atoms refined smoothly using a weighting scheme of unity until the residual had decreased to 0.17. The thermal parameters were then converted to an anisotropic ellipsoidal model and the refinement was continued until the residual converged at 0.090. An examination of the bond distances revealed the phenyl ring to be grossly distorted with the carbon-carbon distances ranging from C(6)-C(7),  $1.21 \text{ \AA}$  to C(9)-C(10),  $1.59 \text{ \AA}$ . To relieve the distortion, the y-coordinates of C(6) and C(10); C(7) and C(9); and the z-coordinates of C(6) and C(7); C(9) and C(10), were averaged. The positions of the



methylene and phenyl hydrogens were calculated (38) using the new set of coordinates for the ring carbon atoms and a carbon-hydrogen distance of  $1.05 \text{ \AA}$ . It was reasoned that the inclusion of these hydrogen atoms in the structure factor calculations would help prevent the reoccurrence of the false minimum during the refinement.

The refinement was continued successfully by the full-matrix least-squares method. The coordinates of the nine remaining hydrogen atoms were determined from a difference Fourier with each hydrogen assigned an isotropic thermal parameter of  $B=6.0 \text{ \AA}^2$ . The refinement was continued using the weighting scheme  $w=1.0$  for  $|F_o| < 20$ ;  $w = (20/|F_o|)^2$  for  $|F_o| > 20$  and allowing all of the parameters for the 31 atoms to adjust. This refinement was terminated at the residual 0.051 as the parameters for the non-hydrogen atoms had converged.

The structural parameters for the other enantiomorph were obtained by inversion of the atomic coordinates through the origin of the unit cell. Anomalous dispersion corrections for sulfur and chlorine were included in a structure factor calculation plus one cycle of least-squares for each enantiomorph using the program NRC-10. The conventional and weighted residuals  $R$  and  $R_w$  for these two calculations are listed in Table 3.4.2.

A Hamilton's test (72) proved that each of the  $R$ -factor ratios were significant at the 99.5% confidence level. The chirality of the asymmetric sulfur atom corresponds to the  $R$  configuration.

Nine additional cycles of least-squares were needed to make the positional and thermal parameters for the  $R$  parameter set converge.



Table 3.4.2

A comparison of the conventional and weighted residuals for the two enantiomorphs before and after the first cycle of refinement.

|  | Original<br>Parameter<br>set | Inverted<br>Parameter<br>set | Ratio<br>$R_S:R_R$ |
|--|------------------------------|------------------------------|--------------------|
| Chirality of sulfur  | R                            | S                            |                    |
| Conventional residual<br>after refinement of<br>the scale factor | 0.058                        | 0.061                        | 1.052              |
| Weighted residual after<br>refinement of the scale<br>factor     | 0.079                        | 0.083                        | 1.050              |
| Conventional residual<br>after 1 cycle of<br>least-squares       | 0.049                        | 0.053                        | 1.081              |
| Weighted residual after<br>1 cycle of least-squares              | 0.068                        | 0.072                        | 1.058              |

During this procedure the weights of several reflections, which showed evidence of systematic errors due to secondary extinction and/or counter paralysis, were modified to bring the value of  $w||F_o| - |F_c||^2$  into agreement with the majority of the data.

In order to accelerate the convergence of the refinement, only a specified fraction of the calculated parameter shifts were allowed. During the seventh cycle a number of parameters which were oscillating about a intermediate set of values were adjusted manually. The final values of the weighted and unweighted residuals were 0.052 and 0.043 for the parameters corresponding to the R





configuration. All parameter shifts were less than 0.1 of the corresponding e.s.d.

Ibers and Hamilton (107) suggest that both enantiomorphs should be refined to convergence before a Hamilton's test (72) on the R-factor ratio is used to determine whether the indicated absolute configuration is statistically valid. However, it was not possible to refine the S parameters set below the residual 0.048 without a rapid divergence of parameter shifts. Since the indicated absolute configuration did not agree with that observed in the previous structure, the experimental procedures were examined to see if a blunder had occurred.

(i) Methanolic solutions were made of several crystals

from the same recrystallization batches as those used to collect the Friedel pairs listed in Table 3.3.7 and the intensity data for this structure. The optical rotation spectrum between 450 and 310 nm. was recorded at 27°C by Mr. K. Oikawa using the Cary Model 60 Recording Spectropolarimeter. The signs and magnitudes of the specific rotations for each sample agreed with the values obtained by Po (85).

(ii) Both sets of intensity data were collected on the same machine using a right-handed orientation matrix. No sign transformations were applied to the Miller indices of reflections, during or subsequent to the intensity measurements.



- (iii) In each structure, the corrections made to the form factors of sulfur and chlorine for the imaginary component of anomalous dispersion were 0.6 and 0.7 electrons respectively.
- (iv) The list of symmetry-related positions used in the calculation of structure factors were identical to those listed in Volume I of the International Tables for X-ray Crystallography (10).

A difference Fourier based on the final calculated structure factors suggested that none of the atoms were misplaced. However, the positions corresponding to the two chlorine atoms and the center of the phenyl ring showed diffuse regions of negative difference electron density ranging from -0.55 to -0.45 e/Å<sup>3</sup>. Smaller regions of negative difference electron density were observed at the positions of the sulfur atom and the carbon atoms of the benzyl moiety. These minima are probably associated with series termination errors and systematic errors in the intensity data arising from the neglect of absorption corrections. The final refined values of the atomic positional and thermal parameters are listed in Tables 3.4.3, 3.4.4 and 3.4.5. The parameter e.s.d's enclosed in parentheses refer to the least significant digit(s). The observed and calculated structure factors are listed as 10X the absolute value in Table 3.4.6.



Table 3.4.3

The final positional parameters of the non-hydrogen atoms of (d)-4-chlorobenzyl-ethylmethysulfonium perchlorate. All quantities are multiplied by  $10^4$ .

| Atom  | x/a     | y/b     | z/c     |
|-------|---------|---------|---------|
| S(1)  | 2624(1) | 1180(1) | 2436(1) |
| Cl(1) | 1482(1) | 6163(1) | 2545(2) |
| Cl(2) | 275(1)  | 1021(1) | 7311(1) |
| C(1)  | 3151(4) | 1648(4) | 4357(7) |
| C(2)  | 3549(4) | 1743(3) | 965(7)  |
| C(3)  | 3351(4) | 1396(3) | -808(7) |
| C(4)  | 1324(3) | 1792(3) | 2158(6) |
| C(5)  | 1348(3) | 2899(3) | 2286(6) |
| C(6)  | 1468(3) | 3434(3) | 832(6)  |
| C(7)  | 1484(4) | 4461(3) | 921(6)  |
| C(8)  | 1400(3) | 4892(3) | 2440(6) |
| C(9)  | 1270(4) | 4371(4) | 3904(6) |
| C(10) | 1226(4) | 3365(3) | 3795(6) |
| O(1)  | 675(3)  | 1989(3) | 7550(6) |
| O(2)  | 650(3)  | 426(3)  | 8639(5) |
| O(3)  | 704(3)  | 643(3)  | 5800(5) |
| O(4)  | -873(3) | 1039(3) | 7254(5) |



Table 3.4.4

The final anisotropic thermal parameters for the atoms whose coordinates are listed in Table 3.4.3. All quantities are multiplied by  $10^4$ .

| Atom  | $U_{11}^*$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{23}$ | $U_{13}$ |
|-------|------------|----------|----------|----------|----------|----------|
| S(1)  | 226(2)     | 160(2)   | 279(3)   | -13(2)   | 15(3)    | -13(3)   |
| Cl(1) | 341(4)     | 207(2)   | 411(4)   | 46(2)    | -36(4)   | -74(4)   |
| Cl(2) | 208(2)     | 183(2)   | 224(2)   | 12(2)    | -17(2)   | 8(2)     |
| C(1)  | 279(14)    | 288(13)  | 282(14)  | 12(11)   | 0(13)    | -41(12)  |
| C(2)  | 208(11)    | 200(10)  | 305(13)  | -35(9)   | -23(11)  | 22(11)   |
| C(3)  | 274(13)    | 224(12)  | 273(13)  | 10(11)   | -9(11)   | -14(11)  |
| C(4)  | 168(9)     | 212(9)   | 271(12)  | -15(8)   | 11(10)   | 4(10)    |
| C(5)  | 142(8)     | 212(9)   | 235(11)  | 11(7)    | 17(9)    | -24(9)   |
| C(6)  | 175(9)     | 236(10)  | 205(10)  | 12(9)    | 5(9)     | -24(9)   |
| C(7)  | 226(12)    | 205(10)  | 232(11)  | 27(10)   | 25(9)    | -35(10)  |
| C(8)  | 192(10)    | 194(8)   | 263(12)  | 50(7)    | -12(10)  | -11(11)  |
| C(9)  | 223(12)    | 312(14)  | 223(12)  | 66(11)   | -43(11)  | 30(10)   |
| C(10) | 237(12)    | 245(11)  | 206(11)  | 35(10)   | 12(9)    | 26(10)   |
| O(1)  | 388(11)    | 243(9)   | 555(16)  | -42(8)   | -65(11)  | -90(14)  |
| O(2)  | 373(12)    | 390(12)  | 350(11)  | 87(10)   | 105(10)  | 47(11)   |
| O(3)  | 425(13)    | 425(13)  | 288(10)  | 21(11)   | -77(10)  | 84(11)   |
| O(4)  | 243(9)     | 356(10)  | 444(13)  | 0(8)     | -135(11) | -35(9)   |

\* These coefficients are defined in the Preface





Table 3.4.5

The final positional and thermal parameters for the hydrogen atoms of (d)-4-chlorobenzyl-ethylmethyisulfonium perchlorate. The hydrogen atoms are numbered by multiplying the atom number of the bonded carbon by 10 and adding 1, 2 or 3.

| Atom   | x/a    | y/b    | z/c     | U <sub>iso</sub> |
|--------|--------|--------|---------|------------------|
| H(11)  | 370(4) | 130(3) | 467(6)  | 93(18)           |
| H(12)  | 314(4) | 245(4) | 465(6)  | 94(18)           |
| H(13)  | 272(4) | 159(3) | 506(6)  | 94(18)           |
| H(21)  | 439(3) | 148(3) | 129(6)  | 67(13)           |
| H(22)  | 342(3) | 254(3) | 117(6)  | 76(14)           |
| H(31)  | 277(3) | 156(3) | -135(5) | 52(13)           |
| H(32)  | 407(3) | 170(3) | -140(6) | 68(13)           |
| H(33)  | 341(4) | 76(3)  | -95(6)  | 78(13)           |
| H(41)  | 92(4)  | 153(4) | 307(7)  | 97(18)           |
| H(42)  | 111(4) | 158(3) | 90(7)   | 83(16)           |
| H(61)  | 144(4) | 315(4) | -38(6)  | 90(17)           |
| H(71)  | 152(4) | 485(3) | -16(6)  | 83(16)           |
| H(91)  | 122(3) | 471(3) | 498(5)  | 70(14)           |
| H(101) | 121(3) | 306(3) | 473(5)  | 70(14)           |





Table 3.4.6

The observed structure amplitudes and calculated structure factors on the absolute scale  $\times 10$  for (d)-4-chlorobenzyl-ethylmethylsulfonium perchlorate. The unobserved reflections are indicated by an asterisk (\*). Reflections which were assigned a modified weight during the final stages of the refinement are designated with a dagger (†).

[illegible]



### 3.4.2 Results and Discussion

The molecular structure of dextrorotatory 4-chlorobenzylethyl-methylsulfonium perchlorate is shown in Figure 3.4.4. The chirality of the sulfonium pole corresponds to the R-configuration. The overall conformation of the cation is very similar to that observed for the structure of the 4-nitrobenzyl homologue. In contrast to the previous two structures, the perchlorate ion has a regular tetrahedral geometry.

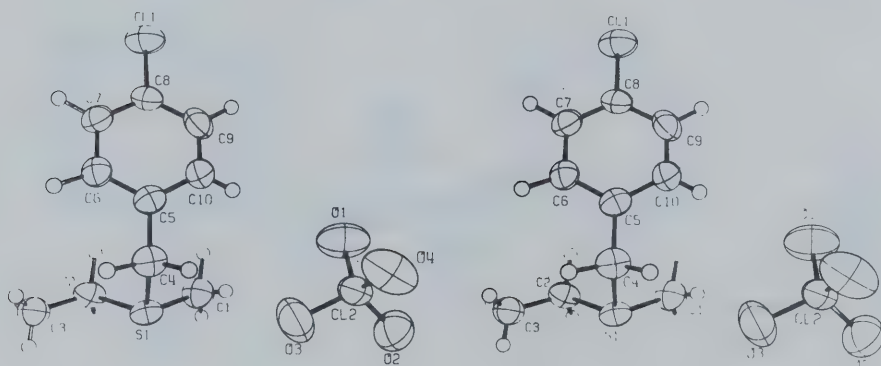


Figure 3.4.4 The molecular structure of (R) 4-chlorobenzylethyl-methylsulfonium perchlorate. The thermal ellipsoids of the anisotropic atoms are scaled to include 35% probability.

Bond distances and bond angles excluding hydrogen atoms are depicted in Figure 3.4.5. The average e.s.d's in the angles SCC, CSC, OClO, ClCC and CCC are 0.3, 0.2, 0.3, 0.4 and 0.4° respectively. Carbon-hydrogen covalent bond distances are listed in Table 3.4.7. The average carbon-hydrogen bond distances is









Table 3.4.7

## Carbon-hydrogen bond lengths

|            |           |              |           |
|------------|-----------|--------------|-----------|
| C(1)-H(11) | 0.87(5) Å | C(3) -H(33)  | 0.88(4) Å |
| C(1)-H(12) | 1.13(5)   | C(4) -H(41)  | 0.95(5)   |
| C(1)-H(13) | 0.78(5)   | C(4) -H(42)  | 1.08(5)   |
| C(2)-H(21) | 1.13(4)   | C(6) -H(61)  | 1.05(5)   |
| C(2)-H(22) | 1.42(4)   | C(7) -H(71)  | 1.03(5)   |
| C(3)-H(31) | 0.89(4)   | C(9) -H(91)  | 0.98(4)   |
| C(3)-H(32) | 1.09(5)   | C(10)-H(101) | 0.85(4)   |

Table 3.4.8

## Bond distances corrected for thermal motion (49)

| Atom A | Atom B | Uncorrected<br>bond length | Lower<br>bound | Riding<br>motion | Upper<br>bound |
|--------|--------|----------------------------|----------------|------------------|----------------|
| S(1)   | C(1)   | 1.787(6) Å                 | 1.788 Å        | 1.799 Å          | 1.919 Å        |
| S(1)   | C(2)   | 1.811(5)                   | 1.811          | 1.815            | 1.932          |
| S(1)   | C(4)   | 1.821(4)                   | 1.821          | 1.822            | 1.938          |
| Cl(2)  | O(1)   | 1.430(4)                   | 1.439          | 1.476            | 1.658          |
| Cl(2)  | O(2)   | 1.417(4)                   | 1.421          | 1.442            | 1.595          |
| Cl(2)  | O(3)   | 1.418(4)                   | 1.422          | 1.445            | 1.609          |
| Cl(2)  | O(4)   | 1.415(4)                   | 1.425          | 1.461            | 1.634          |

the S-C(1) bond. Assuming that the motion of C(1) relative to sulfur is best described by the 'riding motion' approximation, the corrected S-C(1) bond length is 1.799 Å. The model which best describes the motions of C(2) and C(4) relative to sulfur is probably intermediate between one of correlated parallel



Table 3.4.9

A comparison of geometries of the three substituted benzylethylmethylsulfonium cations determined in this chapter.

| (a) Bond                                    | 4-Cl    | 4-NO <sub>2</sub> | 3,5-diCH <sub>3</sub> -4-OCH <sub>3</sub> |
|---|---------|-------------------|---|
| < S-C >                                     | 1.811 Å | 1.810 Å           | 1.806 Å                                   |
| S-C(1) *                                    | 1.799   | 1.803             | 1.800                                     |
| S-C(2) *                                    | 1.813   | 1.806             | 1.815                                     |
| S-C(4) *                                    | 1.821   | 1.822             | 1.803                                     |
| < C(sp <sup>2</sup> )-C(sp <sup>2</sup> ) > | 1.384   | 1.383             | 1.388                                     |
| height of pyramid                           | 0.756   | 0.767             | 0.804                                     |

## (b) Bond angles

|             |        |        |        |
|-------------|--------|--------|--------|
| C(1)-S-C(2) | 100.2° | 100.9° | 101.0° |
| C(1)-S-C(4) | 104.9  | 104.3  | 102.6  |
| C(2)-S-C(4) | 106.0  | 104.3  | 100.8  |
| S-C(2)-C(3) | 111.9  | 111.2  | 110.5  |
| S-C(4)-C(5) | 115.8  | 116.6  | 111.1  |

\* The S-C distances are corrected for thermal motion.



displacements and the 'riding motion' approximation. Using corrected values of  $1.813 \text{ \AA}$  and  $1.821 \text{ \AA}$  for the S-C(2) and S-C(4) bond lengths, the average C-S bond length is  $1.811 \text{ \AA}$ . This agrees with the average C-S bond lengths of  $1.810 \text{ \AA}$  and  $1.806 \text{ \AA}$  observed in the two previous structures.

Table 3.4.9 shows that the C-S-C and S-C-C angles are very similar for the 4-chloro and 4-nitrobenzyl homologues. This similarity corroborates the earlier finding that the geometry of the sulfonium pole is dependent on the number and magnitude of non-bonded steric interactions among the sulfur atom and its three ligands. In particular, the discrepancy between the S-C(4)-C(5) and S-C(2)-C(3) angles is probably because the  $\text{CH}_3\text{-S-C}_2\text{H}_5$  moiety is in Van der Waals contact with the phenyl ring. The steric interactions between C(1) and C(10), C(2) and C(6) result in a compression of the C(1)-S-C(2) angle ( $100.2^\circ$ ) and an enlargement of the angles C(1)-S-C(4) ( $104.9^\circ$ ) and C(2)-S-C(4) ( $106.0^\circ$ ). Short interatomic contacts which are important in the observed geometry of the sulfonium pole are listed in Table 3.4.10.

Table 3.4.10

Short interatomic contacts among the three substituents bonded to sulfur in the 4-chlorobenzylethylmethylsulfonium cation.

| Atom A | Atom B | Separation         | Atom A | Atom B | Separation         |
|--------|--------|--------------------|--------|--------|--------------------|
| S      | C(3)   | $2.76 \text{ \AA}$ | C(1)   | C(5)   | $3.26 \text{ \AA}$ |
| S      | C(5)   | 2.84               | C(1)   | C(10)  | 3.37               |
| S      | C(6)   | 3.64               | C(2)   | C(5)   | 3.31               |
| S      | C(10)  | 3.63               | C(2)   | C(6)   | 3.46               |





The Newman projections down the S-C(2) and S-C(4) bonds for each of the sulfonium cations is shown in Figure 3.4.6.

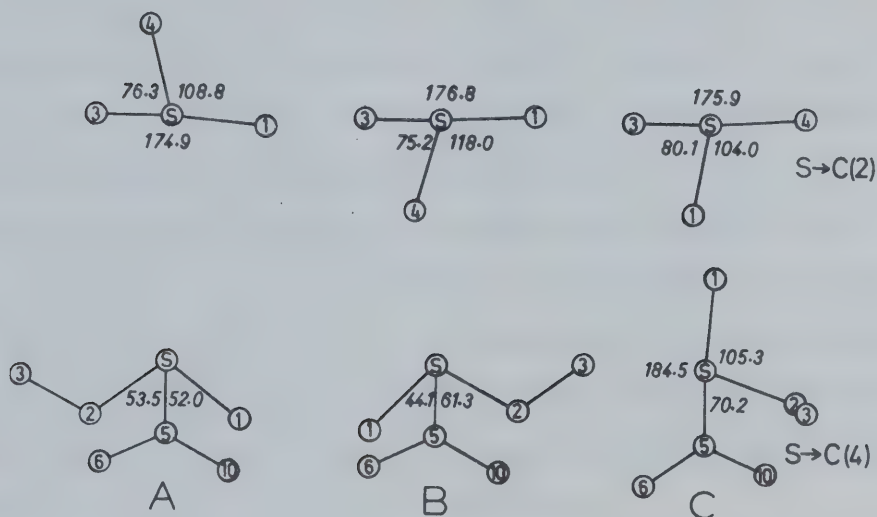


Figure 3.4.6 Newman projections of the S-C(2) and S-C(4) bonds in: A, 4-chlorobenzylethylmethysulfonium perchlorate; B, 4-nitrobenzylethylmethysulfonium perchlorate; C, 3,5-dimethyl-4-methoxybenzylethylmethysulfonium perchlorate.

Whereas a gauche interaction between C(1) and phenyl is possible for both A and B, C(1) is trans with respect to phenyl in C. Also, the C(2)-S-C(4)-C(5) torsion angle is largest for C. A comparison of torsion angles defined by substituents to the S-C(2) atom pair suggests a gauche interaction is possible between C(3) (methyl) and either C(4) or C(1) (methyl).

In each of the three structures all S-C-C angles are greater than 109.5°. Excluding the two large S-C(4)-C(5) angles, the 4



remaining S-C-C angles have an average value of  $111.2^\circ$ .

The direction-cosine equation of the plane defined by the atoms C(1), C(2) and C(4) of the 4-chlorobenzylethylmethysulfonium cation is  $0.0440x - 0.9975y - 0.0561z = -2.6242$ . The distance of the sulfur atom from this plane is  $0.756(1) \text{ \AA}$ . Although the height of the pyramid is largest in the case of the 3,5-dimethyl-4-methoxybenzylethylmethysulfonium cation ( $0.804(1) \text{ \AA}$ ), the first order rate constant for the inversion of that cation (see Table 3.1.1) is larger than for the 4-nitrobenzyl or 4-chlorobenzyl homologues. However, because the last two mentioned cations exhibited a different conformation and show evidence of steric interaction between the three ligands to sulfur, any correlation between pyramid height and the activation parameters is fortuitous.

The C(2)-C(3), C(4)-C(5) and C(8)-C(1) distances of  $1.516(8)$ ,  $1.524(6)$  and  $1.750(4) \text{ \AA}$  are within the normal range of bond lengths expected for each of their particular categories. Reudman (108) reports that the average aromatic  $C(sp^2)$ -C(1) distance (not corrected for thermal motion) is  $1.737(16) \text{ \AA}$ .

Bond angles containing a  $C(sp^2)$  atom at the apex are regular and symmetrical about the C(5)-C(8) direction. Only the C(7)-C(8)-C(9) angle of  $122.8(4)^\circ$  is significantly larger than the theoretical value of  $120^\circ$ . The large value of this angle may be due to the electron-withdrawing properties of the chlorine atom. The corresponding valence angles in chloropheniramine maleate (8) and p,p'-dichlorodiphenyl sulfone (109) are  $120.6(4)^\circ$  and  $121.5(5)^\circ$  respectively.

The average  $C(sp^2)$ -C( $sp^2$ ) bond length of  $1.384(8) \text{ \AA}$  agrees with the average  $C(sp^2)$ -C( $sp^2$ ) distances of  $1.383(5) \text{ \AA}$  and  $1.388(5) \text{ \AA}$



observed in the 4-nitrobenzyl and 3,5-dimethyl-4-methoxybenzyl substituents. However the C(6)-C(7) bond length of 1.413(6) Å is significantly longer than the C(7)-C(8) bond length of 1.355(7) Å. This discrepancy is probably due to systematic errors in the intensity data such as the neglect of absorption corrections. The best least-squares fit of the positional parameters of the six ring carbon atoms to the direction-cosine equation of a plane yielded the equation  $-0.9929x + 0.0293y - 0.1155z = -1.7036$  ( $\chi^2 = 37.4$ ). The exoplanar distances of the ring carbon atoms are C(5), -0.011; C(6), -0.001; C(7), 0.012; C(8), -0.009; C(9), 0.006; C(10), 0.017 Å. The exoplanar distances of those atoms bonded to the ring are C(4), -0.014; Cl(1), -0.067; H(61), 0.14; H(71), 0.08; H(91), -0.03; H(101), -0.06 Å.

The perchlorate ion is in the form of a regular tetrahedron. All O-Cl-O bond angles fall within the limits 107.8(3) and 111.1(3)°. The four Cl-O distances were very similar with an average value of 1.420(3) Å. Riding motion corrections to these bond distances (shown in Table 3.4.8) produced Cl-O bond distances between 1.442 and 1.476 Å and having an average value of 1.456 Å which agrees with the Cl-O distance of 1.46(1) Å reported for nitronium perchlorate (102).

The regularity of the perchlorate ion is readily interpretable from the crystal packing which is shown in projection by Figures 3.4.7 and 3.4.8. The two oppositely charged species are clustered about the screw axis at  $1/4, 0, z$ . There are three short sulfur...oxygen contacts less than 3.7 Å. They are



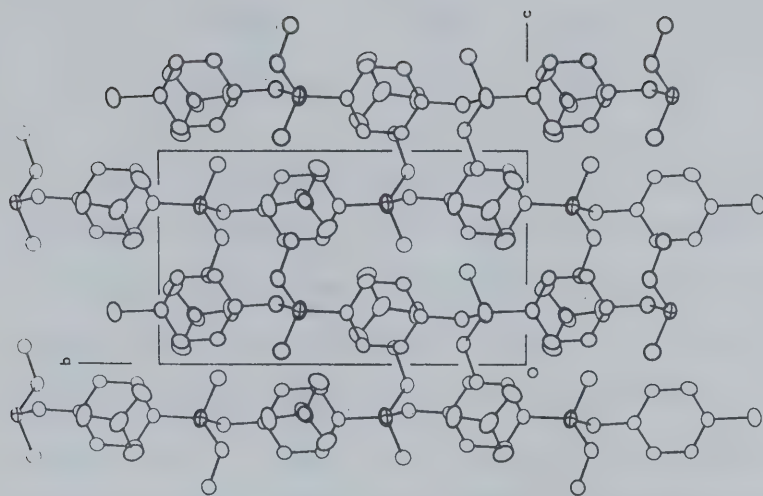


Figure 3.4.8 Projection of the unit cell contents onto (1 0 0)

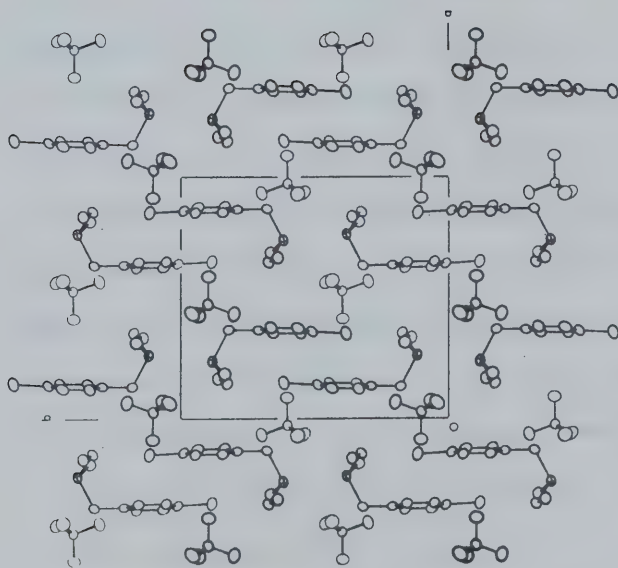


Figure 3.4.7 Projection of the unit cell contents onto (0 0 1).





|   |      |  |        |
|---|------|--|--------|
| S | O(3) | (x, y, z)                                  | 3.66 Å |
| S | O(3) | ( $\frac{1}{2}$ -x, -y, $-\frac{1}{2}$ +z) | 3.50 Å |
| S | O(2) | ( $\frac{1}{2}$ -x, -y, $-\frac{1}{2}$ +z) | 3.21 Å |

The two projection diagrams also show how the interpretation of the Harker sections ( $U, V, \frac{1}{2}$ ) and ( $\frac{1}{2}, V, W$ ) is complicated by a high degree of overlap. In particular, when the unit cell is projected onto the b-c plane, only the atoms C(1), C(2), C(4), C(8) and C(10) do not overlap with other non-hydrogen atoms in the unit cell. Figure 3.4.7 and Figure 3.4.8 indicate that an interchange of the ethyl and methyl substituents would not essentially alter the crystal packing but would change the optic hand of the molecule.

Although one of the original purposes behind determining the crystal structures presented in sections 3.3 and 3.4 was the determination of the absolute configuration of two optically active sulfonium salts, the determined absolute configurations are somewhat contradictory. Although the (R) 4-chlorobenzylethylmethysulfonium cation and the (R) 3,5-dimethyl-4-methoxy-benzylethylmethysulfonium cation exhibit dissimilar conformations, there is no evidence to suggest that each cation does not exhibit a complete freedom of rotation about the S-C(4) or S-C(2) single bonds in solution. If the two cations have a similar absolute configuration and conformation, one would expect their optical rotations to have the same direction at long wavelengths. This however is not the case. Because the parameters corresponding to the two optic hands of each cation were not each refined to convergence, the determination of absolute configuration based on the significance of a Hamilton's test (72)



or a comparison of intensities for Friedel pairs should be accepted with some reservation. Undoubtedly, the best method to determine absolute configuration is to use an internal standard such as the (R) or (S) tartrate ion in the place of the perchlorate ion.



# APPENDIX

This appendix is intended to illustrate some of the details of the work which led to the determination of the structure of ouabain tetrahydrate by the use of direct methods.

## (a) Choice of origin defining reflections.

A detailed description of the rules for the choice of origin defining reflections for the accentric space groups is given by Hauptman and Karle (110). In the space group  $P2_1^2 2_1^2 2_1^2$  the phases of three linearly independent two-dimensional reflections may be specified to have one of two possible values which differ by  $\pi$  radians provided that the following expression is satisfied.

$$\det \text{ modulo } 2 \begin{vmatrix} h_1 & k_1 & \ell_1 \\ h_2 & k_2 & \ell_2 \\ h_3 & k_3 & \ell_3 \end{vmatrix} = \pm 1$$

Two criteria are adhered to in the selection of the origin defining reflections.

- (i) The reflection should have large values of  $|E|$  and participate in a large number of sigma-2 equations. It is preferable that the set of origin defining reflections should generate several new reflection phases without the requirement of additional phase information.
- (ii) One origin defining reflection should be chosen from each of the two-dimensional zones.



(b) Choosing the Enantiomorph

In any space group there exists a class of reflections whose phases are independent of the choice of origin and depend only on the molecular structure. These reflections are called the structure invariants and for the space group  $P2_12_12_1$  are those reflections belonging to the parity group  $eee$ . The form of the structure factor expression for these reflections is:

$$A = 4 \cos(2\pi hx) \cos(2\pi ky) \cos(2\pi lz)$$

$$B = -4 \sin(2\pi hx) \sin(2\pi ky) \sin(2\pi lz)$$

If the molecular structure is replaced by its enantiomorph  $i'$  i.e.,  $x_i' = -x_i$ ,  $y_i' = -y_i$ ,  $z_i' = -z_i$  then by substitution  $A' = A$  and  $B' = -B$ . Thus, in order to specify one of the enantiomorphs, a structure invariant must be identified as having a phase not equal to 0 or  $\pi$ . By arbitrarily specifying the phase of a fourth two-dimensional reflection so that a linear combination of that reflection with one or more of the origin defining reflections defines a structure invariant with a phase of  $\pm \pi/2$ , one of the two enantiomorphs will be chosen.

The development of new reflection phases is carried out with the sigma-2 formula (4) and the tangent formula (5) which are listed in the Preface.

In the case where the new reflection phase is determined from one set of index triplets the process is referred to as an extension. In cases where several sets are used, the value of the new phase is generally improved and the process is referred to as a phase refinement





Table A.1

## Starting Phase Information

| Set 1 |    |    |      |               |  | Set 2 |    |    |      |               |  |
|-------|----|----|------|---------------|--|-------|----|----|------|---------------|--|
|       |    |    | E    | $\phi$        |  |       |    |    | E    | $\phi$        |  |
| 10    | 0  | 7  | 3.55 | $-\pi/2$      |  | 0     | 2  | 11 | 2.79 | 0             |  |
| 0     | 9  | 4  | 2.44 | $\pi/2$       |  | 1     | 0  | 12 | 2.88 | 0             |  |
| 9     | 5  | 0  | 2.35 | $\pi/2$       |  | 4     | 9  | 0  | 2.05 | 0             |  |
| 0     | 2  | 11 | 2.79 | 0*            |  | 10    | 0  | 7  | 3.55 | $-\pi/2^*$    |  |
| 12    | 22 | 0  | 3.52 | 0†            |  | 12    | 22 | 0  | 3.52 | 0†            |  |
| 12    | 0  | 6  | 2.42 | $\pi^\dagger$ |  | 12    | 0  | 6  | 2.42 | $\pi^\dagger$ |  |

\* the enantiomorph

† the reflection phase was determined  
from the sigma-1 formulae (see page 15).

although the term is not to be confused with least-squares refinement.

The choice of the origin and enantiomorph defining reflections was narrowed down to the two sets of reflections given in Table A.1. All reflections with  $|E| > 2.0$ , sorted into the eighty parity groups, are listed in descending values of  $|E|$  in Table A.2.



Table A.2

Reflections with  $|E| > 2.0$ 

| e  | e  | e  | E    | e  | e  | e  | E    | e  | e  | e  | E    | e  | e  | e  | E    |
|----|----|----|------|----|----|----|------|----|----|----|------|----|----|----|------|
| 12 | 22 | 0  | 3.52 | 10 | 0  | 7  | 3.55 | 6  | 11 | 4  | 2.86 | 3  | 26 | 10 | 2.89 |
| 0  | 4  | 8  | 3.14 | 6  | 16 | 3  | 3.11 | 0  | 1  | 12 | 2.55 | 1  | 0  | 12 | 2.88 |
| 0  | 6  | 4  | 2.89 | 0  | 2  | 11 | 2.79 | 4  | 29 | 2  | 2.47 | 7  | 10 | 6  | 2.61 |
| 12 | 2  | 0  | 2.65 | 2  | 24 | 9  | 2.60 | 0  | 9  | 4  | 2.44 | 15 | 16 | 0  | 2.57 |
| 8  | 18 | 10 | 2.57 | 12 | 0  | 7  | 2.55 | 2  | 21 | 10 | 2.40 | 7  | 24 | 6  | 2.38 |
| 4  | 4  | 0  | 2.49 | 2  | 16 | 5  | 2.44 | 8  | 3  | 8  | 2.35 | 9  | 6  | 0  | 2.35 |
| 12 | 0  | 6  | 2.42 | 2  | 0  | 9  | 2.42 | 10 | 13 | 10 | 2.31 | 11 | 16 | 6  | 2.32 |
| 10 | 6  | 0  | 2.41 | 10 | 2  | 7  | 2.36 | 0  | 29 | 6  | 2.31 | 1  | 20 | 10 | 2.30 |
| 0  | 16 | 10 | 2.41 | 8  | 8  | 7  | 2.34 | 16 | 11 | 0  | 2.28 | 9  | 0  | 8  | 2.28 |
| 2  | 6  | 0  | 2.40 | 2  | 26 | 1  | 2.31 | 0  | 3  | 8  | 2.27 | 3  | 28 | 0  | 2.25 |
| 14 | 0  | 0  | 2.30 | 6  | 14 | 3  | 2.29 | 4  | 23 | 2  | 2.22 | 3  | 16 | 6  | 2.25 |
| 0  | 18 | 10 | 2.28 | 2  | 8  | 13 | 2.17 | 8  | 1  | 8  | 2.21 | 11 | 2  | 6  | 2.19 |
| 10 | 2  | 4  | 2.25 | 4  | 34 | 5  | 2.15 | 8  | 9  | 0  | 2.17 | 7  | 16 | 2  | 2.16 |
| 0  | 26 | 10 | 2.24 | 12 | 2  | 7  | 2.15 | 14 | 3  | 0  | 2.16 | 3  | 0  | 12 | 2.14 |
| 4  | 0  | 8  | 2.23 | 0  | 4  | 13 | 2.12 | 4  | 13 | 0  | 2.15 | 1  | 2  | 8  | 2.06 |
| 4  | 8  | 0  | 2.21 | 12 | 24 | 3  | 2.12 | 16 | 13 | 0  | 2.11 | 1  | 4  | 12 | 2.04 |
| 8  | 4  | 0  | 2.18 | 8  | 14 | 7  | 2.03 | 2  | 1  | 12 | 2.10 | 5  | 0  | 8  | 2.03 |
| 6  | 4  | 12 | 2.17 | 2  | 12 | 5  | 2.02 | 6  | 5  | 12 | 2.06 |    |    |    |      |
| 0  | 24 | 10 | 2.13 |    |    |    |      | 4  | 9  | 0  | 2.05 |    |    |    |      |
| 12 | 24 | 2  | 2.08 |    |    |    |      | 0  | 25 | 2  | 2.03 |    |    |    |      |
| 6  | 12 | 8  | 2.06 |    |    |    |      | 4  | 13 | 10 | 2.00 |    |    |    |      |
| 4  | 6  | 0  | 2.01 |    |    |    |      |    |    |    |      |    |    |    |      |

Continued....



Table A.2 (continued)

| e  | o  | o | E    | o  | e  | o  | E    | o  | o  | e  | E    | o  | o  | o | E    |
|----|----|---|------|----|----|----|------|----|----|----|------|----|----|---|------|
| 8  | 7  | 7 | 2.56 | 9  | 10 | 7  | 3.07 | 3  | 27 | 2  | 2.47 | 3  | 9  | 1 | 2.72 |
| 6  | 7  | 7 | 2.51 | 13 | 20 | 1  | 2.88 | 15 | 1  | 0  | 2.32 | 9  | 9  | 7 | 2.64 |
| 10 | 7  | 1 | 2.41 | 1  | 6  | 7  | 2.73 | 7  | 3  | 12 | 2.31 | 13 | 17 | 1 | 2.41 |
| 0  | 7  | 7 | 2.31 | 9  | 2  | 7  | 2.61 | 3  | 1  | 8  | 2.27 | 5  | 11 | 1 | 2.36 |
| 10 | 9  | 7 | 2.16 | 11 | 2  | 5  | 2.57 | 7  | 5  | 8  | 2.24 | 5  | 27 | 9 | 2.34 |
| 10 | 25 | 5 | 2.14 | 13 | 0  | 5  | 2.57 | 7  | 1  | 12 | 2.20 | 3  | 17 | 5 | 2.20 |
| 0  | 5  | 7 | 2.11 | 3  | 20 | 9  | 2.52 | 7  | 13 | 2  | 2.12 | 1  | 3  | 1 | 2.15 |
| 0  | 11 | 3 | 2.07 | 1  | 8  | 3  | 2.46 | 11 | 21 | 2  | 2.10 | 5  | 27 | 3 | 2.07 |
|    |    |   |      | 5  | 4  | 1  | 2.39 | 1  | 3  | 2  | 2.08 | 5  | 9  | 1 | 2.07 |
|    |    |   |      | 1  | 4  | 5  | 2.29 | 7  | 3  | 8  | 2.07 | 15 | 3  | 1 | 2.01 |
|    |    |   |      | 13 | 22 | 3  | 2.28 | 15 | 1  | 6  | 2.06 |    |    |   |      |
|    |    |   |      | 9  | 6  | 1  | 2.27 | 7  | 17 | 10 | 2.02 |    |    |   |      |
|    |    |   |      | 7  | 28 | 3  | 2.26 |    |    |    |      |    |    |   |      |
|    |    |   |      | 1  | 0  | 13 | 2.24 |    |    |    |      |    |    |   |      |
|    |    |   |      | 1  | 2  | 1  | 2.22 |    |    |    |      |    |    |   |      |
|    |    |   |      | 9  | 0  | 9  | 2.17 |    |    |    |      |    |    |   |      |
|    |    |   |      | 5  | 14 | 5  | 2.10 |    |    |    |      |    |    |   |      |
|    |    |   |      | 3  | 10 | 1  | 2.10 |    |    |    |      |    |    |   |      |
|    |    |   |      | 9  | 0  | 7  | 2.09 |    |    |    |      |    |    |   |      |
|    |    |   |      | 9  | 0  | 3  | 2.06 |    |    |    |      |    |    |   |      |
|    |    |   |      | 3  | 4  | 9  | 2.03 |    |    |    |      |    |    |   |      |
|    |    |   |      | 13 | 14 | 1  | 2.02 |    |    |    |      |    |    |   |      |



Table A.3

The manual evaluation of new reflection phases using the sigma-2 equation.

|      |   |          |        |  |                     |        |  |          |
|------|---|----------|--------|--|---------------------|--------|--|----------|
| #1.  | $\frac{10 \ 0 \ \bar{7}}{0 \ 2 \ 11}$       | $-\pi/2$ | #11.   | $\frac{11 \ 2 \ \bar{5}}{\bar{1} \ 0 \ 12}$        | $\frac{\pi}{\pi}$   | 19(a)  | $\frac{10 \ 0 \ 7}{\bar{4} \ 9 \ 0}$             | $-\pi/2$ |
|      | $\frac{10 \ 2 \ 4}{-\pi/2}$                 |          |        | $\frac{10 \ 2 \ 7}{0}$                             |                     |        | $\frac{6 \ 9 \ 7}{\pi/2}$                        |          |
| #2.  | $\frac{1 \ 0 \ 12}{0 \ 2 \ \bar{11}}$       | $0$      | #12(a) | $\frac{11 \ 2 \ 5}{\bar{10} \ 2 \ 7}$              | $\frac{\pi}{0}$     | #19(b) | $\frac{6 \ 11 \ \bar{4}}{0 \ 2 \ 11}$            | $-\pi/2$ |
|      | $\frac{1 \ 2 \ 1}{\pi}$                     |          |        | $\frac{1 \ 4 \ 12}{\pi}$                           |                     |        | $\frac{6 \ 9 \ 7}{\pi/2}$                        |          |
| #3.  | $\frac{10 \ 0 \ 7}{1 \ 2 \ \bar{1}}$        | $-\pi/2$ | #12(b) | $\frac{1 \ 2 \ 1}{0 \ 2 \ 11}$                     | $\frac{\pi}{0}$     | #20.   | $\frac{\bar{6} \ 11 \ 4}{11 \ \bar{2} \ 5}$      | $-\pi/2$ |
|      | $\frac{11 \ 2 \ 6}{\pi/2}$                  |          |        | $\frac{1 \ 4 \ 12}{\pi}$                           |                     |        | $\frac{5 \ 9 \ 1}{-\pi/2}$                       |          |
| #4.  | $\frac{12 \ 22 \ 0}{1 \ \bar{3} \ 1}$       | $0$      | #13.   | $\frac{4 \ 9 \ 0}{10 \ \bar{2} \ 7}$               | $\frac{\pi}{\pi}$   | #21.   | $\frac{6 \ 7 \ 7}{5 \ 9 \ \bar{1}}$              | $0$      |
|      | $\frac{13 \ 20 \ 1}{0}$                     |          |        | $\frac{6 \ 7 \ 7}{0}$                              |                     |        | $\frac{11 \ 16 \ 6}{-\pi/2}$                     |          |
| #5.  | $\frac{12 \ 0 \ 6}{\bar{1} \ 2 \ \bar{1}}$  | $\pi$    | #14.   | $\frac{\bar{1} \ 2 \ \bar{1}}{10 \ \bar{2} \ 4}$   | $\frac{0}{\pi/2}$   | #22    | $\frac{\bar{6} \ 16 \ 3}{9 \ 0 \ 3}$             | $-a$     |
|      | $\frac{11 \ 2 \ 5}{\pi}$                    |          |        | $\frac{9 \ 0 \ 3}{\pi/2}$                          |                     |        | $\frac{3 \ 16 \ 6}{\pi/2 - a}$                   |          |
| #6.  | $\frac{10 \ 2 \ 4}{\bar{4} \ 9 \ 0}$        | $-\pi/2$ | #15.   | $\frac{11 \ 2 \ \bar{5}}{1 \ 0 \ 12}$              | $\frac{\pi}{0}$     | #23.   | $\frac{6 \ \bar{7} \ 7}{6 \ 16 \ \bar{3}}$       | $0$      |
|      | $\frac{6 \ 11 \ 4}{\pi/2}$                  |          |        | $\frac{12 \ 2 \ 7}{\pi}$                           |                     |        | $\frac{0 \ 9 \ 4}{\pi + a}$                      |          |
| #7.  | $\frac{1 \ 2 \ 1}{4 \ 9 \ 0}$               | $\pi$    | #16.   | $\frac{\bar{5} \ \bar{11} \ \bar{1}}{13 \ 20 \ 1}$ | $\frac{\pi}{0}$     | #24.   | $\frac{\bar{6} \ 16 \ 3}{6 \ \bar{11} \ 4}$      | $-a$     |
|      | $\frac{5 \ 11 \ 1}{\pi}$                    |          |        | $\frac{8 \ 9 \ 0}{\pi}$                            |                     |        | $\frac{0 \ 5 \ 7}{-a - \pi/2}$                   |          |
| #8.  | $\frac{11 \ 2 \ 6}{1 \ \bar{3} \ 1}$        | $\pi/2$  | #17.   | $\frac{\bar{4} \ 9 \ 0}{12 \ 2 \ 7}$               | $\frac{\pi}{0}$     | #25.   | $\frac{6 \ 16 \ 3}{9 \ 0 \ \bar{3}}$             | $a$      |
|      | $\frac{12 \ 0 \ 7}{\pi/2}$                  |          |        | $\frac{8 \ 7 \ 7}{\pi}$                            |                     |        | $\frac{15 \ 16 \ 0}{a - \pi/2}$                  |          |
| #9.  | $\frac{10 \ 2 \ 4}{\bar{6} \ 11 \ \bar{4}}$ | $-\pi/2$ | #18(a) | $\frac{12 \ 0 \ 7}{\bar{8} \ 7 \ \bar{7}}$         | $\frac{\pi/2}{\pi}$ | #26(b) | $\frac{\bar{8} \ 7 \ 7}{11 \ \bar{2} \ \bar{6}}$ | $0$      |
|      | $\frac{4 \ 13 \ 0}{\pi}$                    |          |        | $\frac{4 \ 7 \ 0}{-\pi/2}$                         |                     |        | $\frac{3 \ 9 \ 1}{\pi/2}$                        |          |
| #10. | $\frac{10 \ 2 \ 4}{6 \ 11 \ \bar{4}}$       | $-\pi/2$ | #18(b) | $\frac{10 \ 0 \ \bar{7}}{\bar{6} \ 7 \ 7}$         | $-\pi/2$            | #26(b) | $\frac{6 \ \bar{7} \ 7}{\bar{3} \ 16 \ \bar{6}}$ | $0$      |
|      | $\frac{16 \ 13 \ 0}{\pi}$                   |          |        | $\frac{4 \ 7 \ 0}{\pi/2}$                          |                     |        | $\frac{3 \ 9 \ 1}{\pi/2 - a}$                    |          |





A manual evaluation of new reflection phases using the sigma-2 equation and starting set #2 is given in Table A.3. The reflection (6 16 3) ( $|E| = 3.11$ ) was given the symbol  $\underline{a}$  after relation #21. This was not absolutely necessary but was done in order to avoid using reflections with  $|E| < 2.0$ . The value of  $\underline{a}$  was indicated to be 0 (or  $\pi$ ) from relations #24, #25, and #26. This is contradicted by relation #23 since (0 9 4) must have a phase of  $\pm \pi/2$ . Similarly the relations #18 are invalid since  $\emptyset$  (4 7 0) is restricted to be 0 or  $\pi$ . Further manual evaluation of the sigma-2 equation led to a large number of contradictions and left the phases of several reflections with large  $|E|$  undetermined.

Similar difficulties were encountered using starting set #1 except that more unknown symbols were needed to continue the hand phasing. This set proved to be less useful because it did not lead to an early determination of  $\emptyset$  (1 2 1).

Several unsuccessful attempts were made to determine reflection phases by means of an iterative application of the tangent formula using the DP-4 phase refinement program of Huber and Brisse (35) and the two starting sets of seven reflection phases (including (6 16 3) = 0,  $\pi$ ). In both cases this process was at a disadvantage due to the inability to weight certain reflection triplets. This was unfortunate in that in many cases the order of acceptance of reflection phases may have a crucial effect on the values of the derived phases. Karle (111) has suggested that reflection triplets in which each member is from the same two-dimensional zone, often violate the sigma-2 equation. For this reason all reflection triplets of this kind were removed from the sigma-2 listing. Several attempts were made to augment the initial sets of phases by the addition



of the reflection phases  $\phi(0\ 6\ 4) = 0, \pi$  and  $\phi(0\ 4\ 8) = 0, \pi$ . Four separate tangent refinements were performed with E-threshold values of 1.9, 1.8 and 1.7. The value of the R-Karle used as a measure of the acceptability of the refined set of phases was greater than 0.26 for each attempt. The resultant E-maps contained a large number of peaks but a partial structure could not be identified.

A different approach was resorted to when it was realised that the values of many of the reflection phases were dependent on the initial values of  $\phi(1\ 2\ 1)$  and  $\phi(10\ 2\ 4)$ . The occurrence of the triplet  $\phi(9\ 0\ 3) \simeq \phi(10\ 2\ 4) + \phi(-1\ 2\ -1)$  suggested that the two general reflections would have phases separated by  $\pi/2$  radians. The value of each phase could be within  $\pi/6$  of the estimates derived in relationships #1 and #2 of Table A.3.

Four sets of phase refinement were performed, the details of which are given in Table A.4. In each calculation, E-threshold limits were 1.9, 1.8, 1.71, and 1.71; the minimum allowed value of  $|E_c|$  was 0.4 and phase oscillation limits were set to  $0.7\pi$  and  $0.4\pi$  for centric and acentric reflections respectively.

A E-map calculated with 317 reflections with  $|E| > 1.71$  and phased during Trial 1 revealed the non-hydrogen atoms of the ouabain skeleton. In the initial attempt  $\phi(10\ 0\ 7)$  was specified as  $\pi/2$  which resulted in the mirror image of the structure. However for purposes of clarity  $\phi(10\ 0\ 7)$  has been specified as  $-\pi/2$  throughout the Appendix.



Table A.4

Four tangent refinement trials using  
different starting phases for (6 16 3),  
(1 2 1) and (9 0 3).

|                       | Trial 1  | Trial 2  | Trial 3  | Trial 4  |
|-----------------------|----------|----------|----------|----------|
| $\emptyset$ (10 0 7)  | $\pi/2$  | $\pi/2$  | $\pi/2$  | $\pi/2$  |
| $\emptyset$ (0 2 11)  | 0        | 0        | 0        | 0        |
| $\emptyset$ (1 0 12)  | 0        | 0        | 0        | 0        |
| $\emptyset$ (4 9 0)   | 0        | 0        | 0        | 0        |
| $\emptyset$ (12 22 0) | 0        | 0        | 0        | 0        |
| $\emptyset$ (12 0 6)  | $\pi$    | $\pi$    | $\pi$    | $\pi$    |
| $\emptyset$ (1 2 1)*  | $7\pi/8$ | $7\pi/8$ | $9\pi/8$ | $9\pi/8$ |
| $\emptyset$ (10 2 4)* | $3\pi/8$ | $3\pi/8$ | $5\pi/8$ | $5\pi/8$ |
| $\emptyset$ (6 16 3)* | 0        | $\pi$    | 0        | $\pi$    |
| $R_{K1}( E  > 1.9)$   | 0.404    | 0.416    | 0.396    | 0.391    |
| $R_{K2}( E  > 1.8)$   | 0.291    | 0.340    | 0.284    | 0.314    |
| $R_{K3}( E  > 1.71)$  | 0.188    | 0.284    | 0.290    | 0.304    |
| $R_{K4}( E  > 1.71)$  | 0.189    | 0.314    | 0.302    | 0.294    |

\* These phases were kept constant during the first refinement cycle only.



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